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BULLETIN OF EXPERIMENTAL BIOLOGY AND MEDICINE

БЮЛЛЕТЕНЬ ЭКСПЕРИМЕНТАЛЬНОЙ БИОЛОГИИ И МЕДИЦИНЫ

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THE INTERACTION BETWEEN EXTINCTIVE AND SUPRALIMINAL INHIBITION

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Original article submitted August 14, 1959

In our previous work on the study of the interaction between supraliminal inhibition and various types of internal inhibition, we investigated the effect of increasing the volume of the positive and differential tones on the higher nervous activity of a dog with a strong type of behavior (Inga). It was found that supraliminal inhibition strengthens differential inhibition (undergoes summation with it). We also observed that when the inhibiting stimulus becomes louder, differentiation becomes more precise (of "zero" pattern), and when the positive stimulus increases in volume, the differential inhibition is primarily disturbed.

The present work was devoted to the study of the interaction between extinctive and supraliminal inhibition.

In I.P. Pavlov's opinion, the process of extinction of conditioned reflexes includes components of conditioned and components of unconditioned inhibition. Of special interest to our investigation are the views on the mechanism of extinction expressedby S.V. Kleshchov, V.F. Pleshkov and others [2]. These authors divided the process of extinction into a number of phases: a rise of the curve of secretion of saliva, followed by a sharp fall in the curve and a residual secretion that is slowly extinguished at a low level. These authors, and also G.V. Skipin [8] and V.K. Fedorov [11], considered that the sharp fall in secretion of saliva during extinction is due to overexcitation of the cortical cells, and consequently to the intervention of supraliminal inhibition.

Because of these views it was important to investigate the process of extinction of a conditioned reflex to an excessively strong stimulus, for in response to the action of such a stimulus the process of conditioned excitation must be more or less restricted in its intensity by the development of supraliminal inhibition. Should this stimulus by change not be reinforced, the developing extinctive inhibition must interact to some extent with this supraliminal inhibition. Since the process of extinction itself, according to the point of view stated above, includes elements of overexcitation (i.e., of a supraliminal nature), it might be expected that as a result of this "double overexcitation" (from the excessively strong stimulus and the process of extinction) new facts would be obtained which would lead to a better understanding of the processes under study.

EXPERIMENTAL METHOD AND RESULTS

Experiments were carried out on a dog (Al'ma) with a strong type of nervous system, by the method of conditioned salivary food reflexes. Conditioned reflexes were established in the dog to a buzzer, to a pure sound of 400 cps (from an ordinary ZG-10 sound generator), a light and a metronome. A sound of intensity 50 db was used twice (in second and fourth places) in the system of the conditioned reflexes. The work was carried out in a soundproof chamber, and the secretion of saliva was recorded by the Ganike-Kupalov method (one scale division corresponds to 0.01 ml). Alimentary reinforcement was by means of 20 g of a meat and biscuit powder, diluted 1:1 with milk.

After consolidation of the conditioned reflexes (see their mean values in the table), the tone used twice in the system of stimuli was intensified to 122 db. The increase in the loudness of the tone caused an increase in all the conditioned reflexes (see table).

It must be pointed out that this dog was perfectly trained to the action of loud conditioned sound stimuli, and for this reason, when the intensity of the tone was increased, we did not obtain supraliminal inhibition in its "pure" form; from certain indirect signs, however, it could be assumed that this took place when the tone was intensified to 122 db (the maximum of which our amplifier was capable).

It can be seen from the table that the conditioned reflex to the excessively strong tone was smaller on the average than the conditoned reflexes to the weak tone and the buzzer. In many experiments the pattern of secretion of saliva in response to a very loud tone was distorted, i.e., instead of an increase taking place at the moment of reinforcement, the secretion of saliva decreased in response to the tone. In experiments 720, 721, 723, 724, etc., for example, when the secretion was recorded every 5 seconds, the values of the secretion of saliva were as follows (after a lag of 15 seconds): 10, 25, 12; 10, 25, 15; 5, 10, 8; 3, 20, 15. This pattern of secre-

TABLE. Mean Value (of 5 Experiments) of the Conditioned Reflexes Before and After Increasing the Loudness of the Tone to 122 db in the Dog Al'ma

Conditioned stimuli	Magnitude of reflexes before intensification of tone (in scale divisions)		Magnitude of reflexes after intensification of tone II (in scale divisions)		Change in magnitude of reflexes (as % of original)
	experiment		experiment		average of 10 experiments
	700-713	714-718	733-737	742-746	
Buzzer	45.4	49.8	61.0	59.6	+ 25
Tone I (50 db)	33.8	34.2	61.2	67.2	+ 89
Light	33.4	28.2	48.4	50.0	+ 60
Tone II	31.6	31.2	54.4	54.2	+ 73
Metronome	29.2	35.8	53.8	35.6	+ 37

Note. The tone was first increased in intensity in experiment 719.

tion in response to an excessively loud tone was often observed also in subsequent experiments. These facts, in our opinion, suggest that in this case we are dealing with the intervention of supraliminal inhibition in the process of conditioned excitation during the action of an excessively strong stimulus. Further support for this hypothesis is given by the fact that, in the first place, as the excessively loud tone is applied, the ensuing conditioned reflex to the metronome is considerably decreased (the presence of successive inhibition); secondly, when tone I and not tone II in the system was increased in intensity for a period of 5 days, the reflex in response to it fell on the average to 54 scale divisions, which corresponds very closely to the magnitude of the reflex to tone II. Consequently, the magnitude of the last reflex was less than that of the reflexes to the buzzer and tone I, not on account of the position of the stimuli in the stereotype but because of the effect of supraliminal inhibition.

As a preliminary step, before increasing the intensity of the tone, we carried out a sharp, continuous extinction of the conditioned reflexes to the tone (50 db), the light and the buzzer (until three "zeros" had been recorded), and obtained the extinction curves illustrated in Fig. 1.

After increasing the loudness of the tone, we again extinguished the buzzer, tone I (50 db) and tone II (122 db). The process of extinction of the reflexes to the first two stimuli was unchanged (Fig. 2), and extinction of the conditioned reflex to a very loud tone took place far more slowly, as a result of the prolongation of the second and third phases. Under these circumstances the animal showed an unusually intensive motor alimentary reaction (the dog thrust its mouth several times during the time of action of the excessively loud tone, into the feeding bowl, sniffed at it, licked it and so on). The first phase (the rise of the saliva secretion curve) in these cases was either absent or very ill-defined (Fig. 3).

Extinction of the excessively loud tone caused no changes in the magnitude of the other conditioned reflexes, but after subsequent extinction of the conditioned

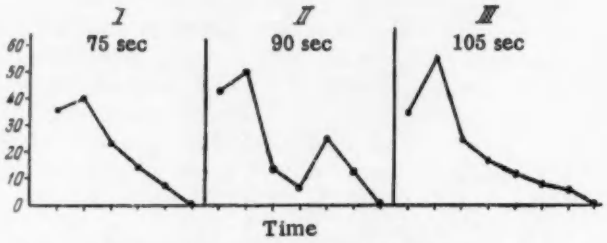

Fig. 1. Sharp continuous extinction of the conditioned reflexes to a stimulus of normal strength (medium – tone and buzzer; weak – light). I) Tone; II) buzzer; III) light. Along the axis of the ordinate – magnitude of secretion of saliva in scale divisions (1 division = 0.01 ml); along the axis of the abscissae – time from beginning of action of the stimulus (each division corresponds to 15 seconds).

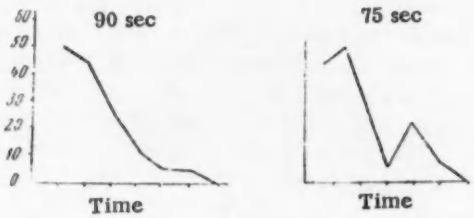

Fig. 2. Sharp continuous extinction of the conditioned reflexes to a buzzer (I) and a tone of intensity of 50 db (II) after increasing the intensity of the second tone to 122 db. Legend as in Fig. 1.

stimulus of normal strength, the process of extinction was altered: It was prolonged to 150 seconds. On the next day, however, extinction of the conditioned reflex to the same stimulus (metronome) occurred at the usual rate (in 90 seconds).

Analysis of the curves of extinction of the conditioned reflexes shown in Fig. 1 indicates that the conditioned reflex to a weak stimulus (light) was extinguished

rather more slowly (on account of the prolongation of the third phase) than the conditioned reflexes to stimuli of medium strength (105, 75 and 90 seconds, respectively); the first phase was most pronounced during extinction of a weak stimulus. The reflex to an excessively strong stimulus (150 seconds; see Fig. 3) was extinguished even more slowly than the conditioned reflex to light. In this case the second phase of extinction was prolonged in addition to the third phase; the first phase was rather ill-defined.

According to the views of V.K. Fedorov [11], P.S. Kupalov [4], and others, regarding the mechanism of extinction, it may be postulated that the extinction of any conditoned reflex involves the participation of supraliminal inhibition, arising as the result of an increase in the level of excitation of the cortical cells at the moment of reinforcement by food and of summation of the stimuli from the conditioned agent and of the conditioned reflex to the time of taking food. Normally (with reinforcement of conditioned stimuli) supraliminal inhibition does not arise, for these processes are inhibited by the act of eating [1, 7, 9 and others]. The facts that we have obtained may thus be explained as follows. The action of a weak conditioned stimulus causes a less intensive excitation than a conditioned stimulus of medium intensity. After extinction of the weak stimulus the level of excitation may rise before the limit of functional capacity of the cortical cells is reached. This was shown in our experiments by the sharp rise in the curve of secretion of saliva in the first phase after extinction of the conditioned reflex to light.

The presence of "reserves of functional capacity" leads to the fact that a weaker supraliminal inhibition develops than after extinction of the conditioned reflexes to stimuli of medium intensity; in the latter case a fairly intensive process of excitation develops, which rapidly reaches its limit after extinction of the conditioned stimulus. Firstly, this prevents a significant increase in the secretion of saliva in the first phase, and secondly, it causes a more marked supraliminal inhibition. On extinction of the reflex in response to an excessively strong stimulus, the conditioned reflex itself (before its extinction) shows a fairly intensive supraliminal inhibition, which is still further intensified during extinction at the moment of reinforcement. As a result of this, an overstraining of the supraliminal inhibition takes place, which is expressed predominance of the process of excitation in the second and third phases and by irradiation of the excitation to the motor area of the cerebral cortex.

An analogous phenomenon during overstraining of supraliminal inhibition in the case of a considerable increase in the intensity of the differential stimulus was observed by Z.V. Troshikhina [10], Mei Chên-T'ung [5] and N.N. Kudryavtseva [3]. Consequently, overstraining of supraliminal inhibition during the extinction of a conditioned reflex to an excessively strong stimulus (in the second phase of extinction) causes a disturbance (weakening) of the internal inhibition produced (third phase of extinction). The prolongation of the phase of residual secretion of saliva during the action of the metronome, observed after extinction of the excessively strong tone, may be attributed to a temporary weakening of the inhibition caused by overstraining of the nervous processes during extinction of the excessively strong stimulus.

SUMMARY

Interaction between extinctive and supraliminal inhibition was studied on dogs by the method of conditioned salivary reflexes. In extinction of a conditioned reflex to an excessively strong stimulus, there occurs an overstraining of the subliminal inhibition, the sequence of which is: predominance of excitation; disinhibition of the extinction process; and irradiation of excitation to the motor area of the cerebral cortex. The conditioned reflexes in response to stimuli of different strength are arranged by the rate of extinction in the following order: Conditioned reflexes to stimuli of moderate strength are the first to be extinguished; the next are those in response to weak conditioned stimuli, and the last, to excessively strong stimuli. Extinction of conditioned reflexes (Phase 1-the rise of the curve of salivary secretion) was most pronounced in extinction of the reflex to the weak stimulus (light), less pronounced in extinction of the reflex to the moderately strong stimuli (metronome, buzzer, 50-db tone), and very weak or even absent in extinction of the reflex to the excessively strong stimulus (122-db tone).

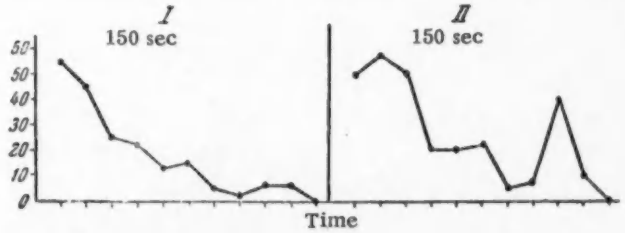

Fig. 3. Sharp continuous extinction of the conditioned reflex to an excessively loud tone (122 db). I) Experiment 732 (January 21, 1959); II) experiment 738 (January 29, 1959). Legend otherwise as in Fig. 1.

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EXTINCTION AND DIFFERENTIATION OF CONDITIONED REFLEXES INDUCED AGAINST A BACKGROUND OF UNCONDITIONED STIMULATION

(Analysis of the Mechanism of the Development and Localization of Internal Inhibition During Covering)

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(Presented by Active Member AMN SSSR P.S. Kupalov)
Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 50, No. 7, pp. 7-12, July, 1960
Original article submitted October 21, 1959

In a previous paper [9] we showed that a complete and stable motor-defensive conditioned reflex may be induced in dogs not only by the usual combinations of stimuli, but also against a background of unconditioned stimulation, with what is known as covering with intensification. An indifferent stimulus, when repeatedly combined with intensification of an existing current, acquires the power in the first place to intensify sharply the motor-defensive reaction of a dog in response to preceding background electrical stimulation of the skin, and secondly to induce a conditioned reflex when used in isolation.

In order to continue the study of the properties of conditioned reflexes to intensification induced by this means, in the present research we investigated the pattern of development of differentiation and extinction of these reflexes.

EXPERIMENTAL METHOD

Experiments were carried out on three dogs (Dik, Sil'va and Ryzhaya), in which a number of conditioned reflexes were induced before the beginning of the present research by means of the covering with intensification technique. In contrast to the usual methods of covering, with covering with intensification the strength of the acting current was increased intermittently (1.5-2.0 times) in each combination immediately after the addition of the conditioned stimulus to the unconditioned (5 seconds after its start). During the extinction or differentiation of these reflexes, we discontinued the intensification of the acting current in conjuction with the use of the inhibiting stimulus against the background of the unconditioned reflex (i.e., we began to cover by the usual method). The source of current was a rectangular impulse generator (70 cps, duration of impulse 1 msec). To record the limb movements we used Petropavlovskii's "knee-caps", connected pneumatically to a Marey's capsule. The animal's respiration was recorded throughout the experiment.

EXPERIMENTAL RESULTS

Formation of differentiation against a background of unconditioned stimulation. At the beginning of the experiments a conditioned reflex to a tone of 1000 cps was induced in the dog Dik by the covering with intensification technique. We attempted to form differentiation between this stimulus and a tone of 200 cps.

The positive stimulus – a tone of 1000 cps (T-1000) – on each application against a background of electrical stimulation of the skin caused intensification of the dog's motor reaction (Fig. 1a; 33nT-1000, 34nT-1000), and we always accompanied this effect by doubling the strength of the acting current. The same positive effect as with T-1000 was produced in the dog Dik by other conditioned stimuli (bell, M-120, light), also when subjected to covering with intensification (see Fig. 1a and b; tests 1n3, 3nM-120, 3nC).

The first application of the tone of 200 cps (T-200) against a background of electrical stimulation of the skin caused no appreciable change in the unconditioned reflex; in the course of the second covering, in response to T-200 the dog developed a powerful orientational reaction, accompanied by weakening of the unconditioned defensive reflex (see Fig. 1a; 2nT-200). In subsequent experiments this difference between the effects of T-1000 and T-200 became obliterated. Both stimuli gave a positive effect (see Fig. 1b; 40nT-1000 and 6nT-200), in spite of the fact that the action of T-1000 was always reinforced (was accompanied by intensification of the current), whereas the application of T-200 against a background of electrical stimulation of the skin was left unreinforced (the current strength remained unchanged throughout the whole combination).

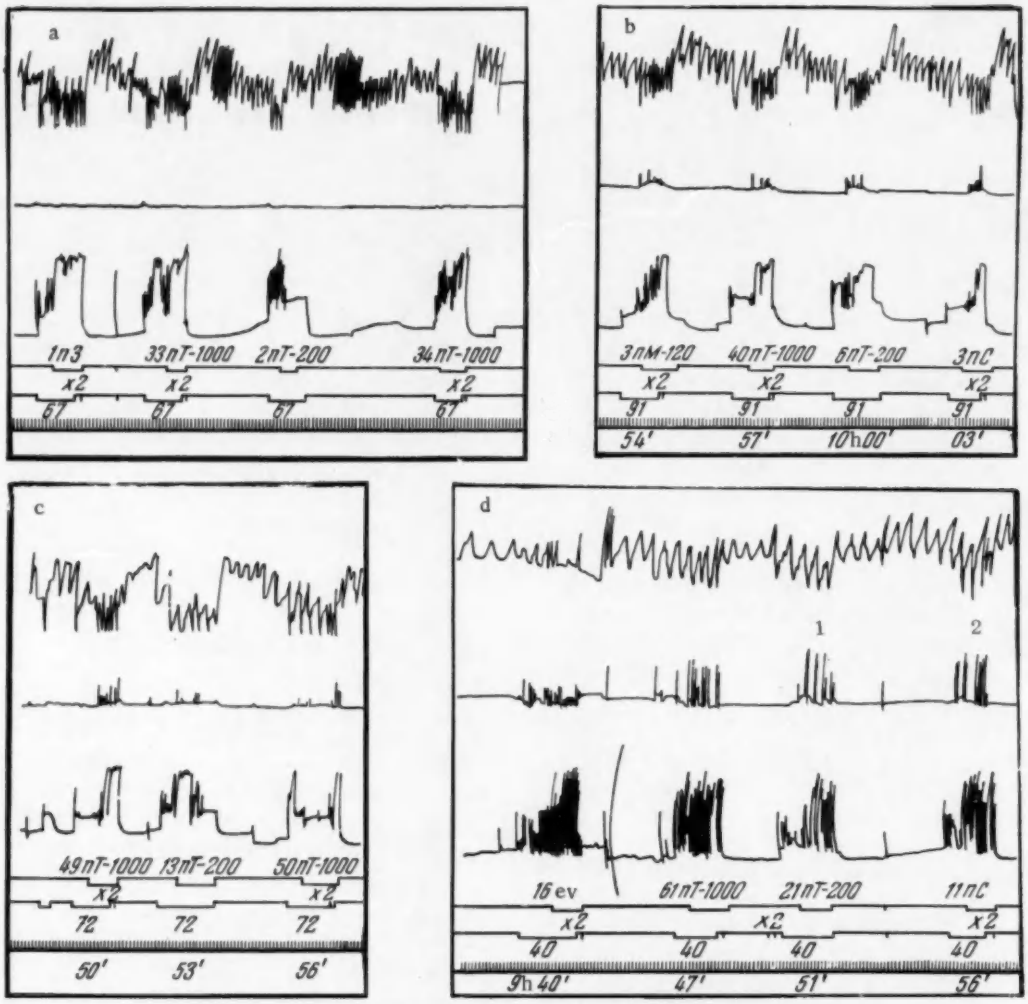

Fig. 1. Differentiation of stimuli against a background of unconditioned stimulation. First experiments. Tone of 1000 cps (T-1000), applied against a background of unconditioned stimulation, is always accompanied by intensification of the acting current; the tone of 200 cps is applied without intensification. The dog Dik. a) Experiment 208 (November 30, 1958); b) experiment 210 (October 3, 1958); c) experiment 214 (October 21, 1958); d) experiment 219 (November 11, 1958). Significance of the curves (from above, down): Pneumogram; movement of the left (unstimulated) hindlimb; movement of the right hindlimb (stimulated by the current); marker of conditioned stimulation; marker of unconditioned stimulation; time marker (1 second). 1) Vocal reaction 2) voice.

The first signs of differentiation between the stimuli appeared after the 12th absence of reinforcement of the 200 cps tone (T-200). In test 13nT-200 (Fig. 1c) this stimulus caused an obvious weakening of the unconditioned reflex instead of an intensification. The immediately ensuing reflex to the positive tone of 1000 cps showed successive inhibition (50nT-1000). The inhibitory action of T-200, however, was unstable and after a short time it again gave way to a positive action (Fig. 1d; 21nT-200). Only after 32 nonreinforcements did the inhibitory effects of T-200 begin to acquire a more stable character, and, in some cases, moreover, after application of the differential stimulus, successive inhibition of the reflex to the positive tone also developed (Fig. 2a; 33nT-200, 67nT-1000).

A further ten nonreinforcements were required for the differentiation to be stable and concentrated. The final results of these experiments are shown in Fig. 2b.

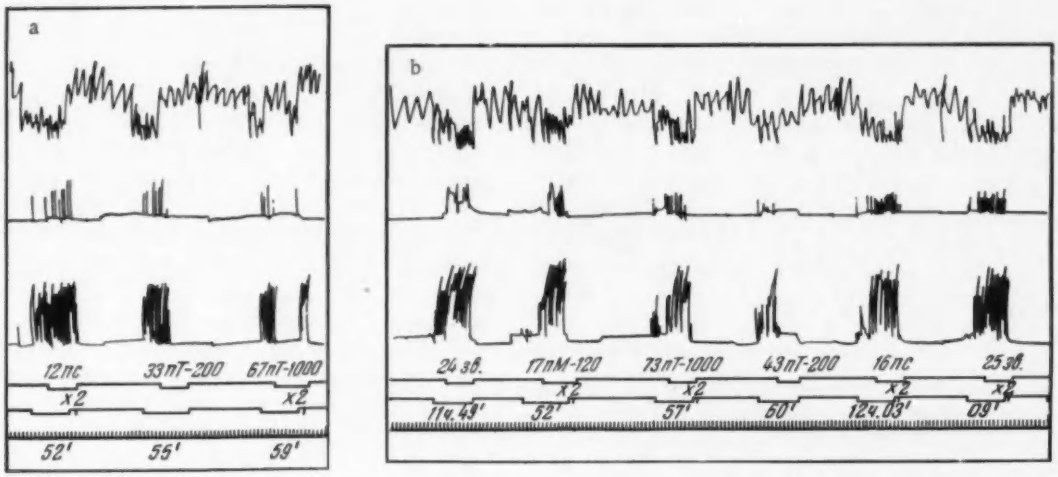

Fig. 2. Complete differentiation of stimuli against a background of unconditioned stimulation. Continuation of experiments on the dog Dik. a) Experiment 221 (November 13, 1958); b) experiment 225 (November 19, 1958). Significance of the curves the same as in Fig. 1.

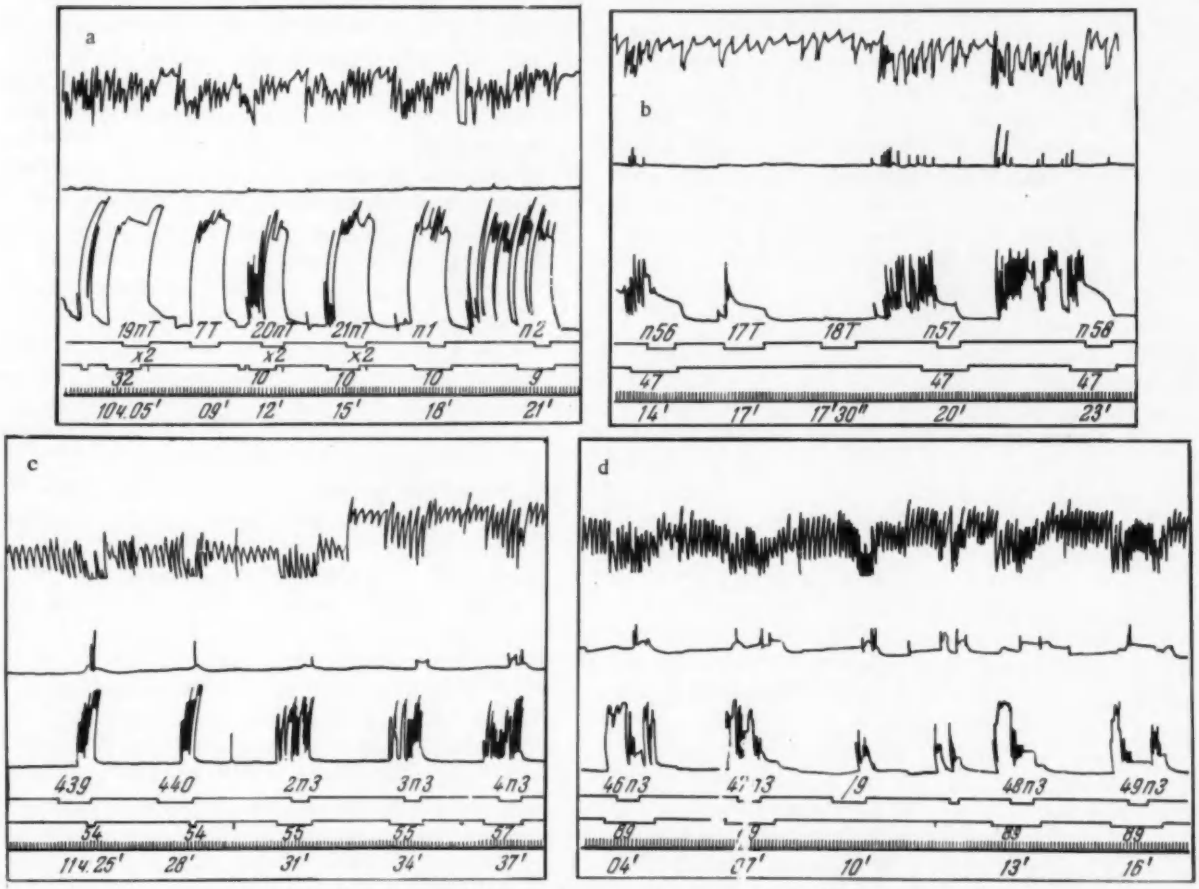

Fig. 3. Extinction of a conditioned reflex against a background of unconditioned stimulation. a) The dog Sil'va, experiment 160 (September 25, 1958); b) the same dog, experiment 167 (September 19, 1958); c,d) the dog Dik, experiment 194 (July 8, 1958) and experiment 198 (July 8, 1958). Significance of the curves as in Fig. 1.

It will be seen that all the positive stimuli—the bell (24), metronome (17nM-120), the tone (73nT-1000), light (16nC) and bell (25)—caused an obvious strengthening of the defensive reaction of the dog, and of both its motor and respiratory components. In contrast to this, the application of the differential stimulus (43nT-200) resulted in sharp inhibition of the unconditioned reflex, in spite of the contining electrical stimulation of the skin.

Extinction of the conditioned reflex to intensification. We obtained the same results in principle by extinction of the conditioned reflexes against a background of unconditioned stimulation as in the experiments to induce differentiation. In the dog Sil'va, before extinction, the positive stimulus—the tone, constantly accompanied by intensification of the acting current—always caused an increase in the strength of the unconditioned reflex. The dog responded to the isolated application of this stimulus with a strong conditioned defensive reaction (Fig. 3a; 7T). After 21nT (see Fig. 3a) we ceased to reinforce the positive effects of the tone by intensification of the current (n1, n2 and so on), i.e., we changed over to the ordinary method of covering. At first this led to a gradual weakening of these effects, and later to their distortion: addition of the tone to the electrical stimulation of the skin began to cause obvious inhibition of the unconditioned reflex (Fig. 3b; n56, n57, n58). It is interesting that these inhibitory effects appeared before the covered stimulus completely lost its power to give rise to a conditioned motor reaction when acting in isolation (see Fig. 3b; 17T). Complete inhibition of the conditioned reaction took place only after 80 "coverings" without intensification. We obtained a similar effect in experiments on the dog Ryzhaya.

The results described above are of interest from several points of view. Firstly, they show that during conditioned stimulation the cerebral cortex retains the ability not only to detect the action of an indifferent or a signal stimulus [1, 6, 7], but also to differentiate between these stimuli (cf. [3]). Secondly, the fact that the unconditioned reflex is inhibited under the influence of an added differential or extinctive stimulus is an important piece of evidence in the doubtful and often debated problem of the spatial localization of internal inhibition. It becomes clear that this inhibition is not localized at the "point" of the conditioned stimulus, nor in the intermediate elements of the conditioned reflex arc, but evidently in those of its links which are directly connected with the performance of the unconditioned reflex; it is probable that the reticular formation of the brain stem takes part in the production of this inhibitory effect [10]. Finally, the results obtained are important in the understanding of the mechanisms of inhibition during covering of conditioned reflexes induced by the usual method. We must dwell on this problem in rather more detail.

We have seen above that the temporary nervous connection formed by the method of covering with intensification is invariably inhibited on the change-over to ordinary covering. The reason for the development of internal inhibition in this case is clear: The positive conditioned effects caused by signal stimuli against a background of an unconditioned reflex cease to be reinforced by a corresponding intensification of the acting current during ordinary covering, and are thus extinguished as nonreinforced. As is easily shown, however, similar relationships also arise during repeated covering of a conditioned stimulus, brought about in the ordinary way. The use of such a conditioned agent against a background of electrical stimulation of the skin in most cases also caused intensification of the unconditioned reflex [2, 6], and the strength of the acting current is not increased under these circumstances. The effect of the conditioned stimulus thus remains unreinforced, and this must inevitably lead to its inhibition [8]. The experiments described below appear to us to confirm this supposition.

The pattern of inhibition developing in covering of a conditioned reflex produced in the usual manner. This series of experiments was carried out on the dogs Dik, Sil'va and Ryzhaya before the use of covering with intensification was begun in these animals. In the usual combinations a bell acted in isolation for 8 seconds, and then to it was added electrical stimulation of the skin for 2 seconds, after which both stimuli were discontinued stimultaneously. During covering, the bell was added to the unconditioned reflex 5 seconds after its onset.

In Fig. 3c we show the results of the first experiment on the dog Dik. We began to cover the conditioned reflex to a bell (tests 439 and 440) with unconditioned stimulation. At first each application of the bell against the background of action of the current (2n3 and later) resulted in an obvious strengthening of the unconditioned defensive reflex—an increase in the frequency and strength of flexion of the stimulated limb, the appearance of movements in the opposite hindlimb, some increase in the strength and rate of respiration and, in some cases, a vocal reaction—the whining or barking of the dog. Later, however, as the covering was repeated, these positive effects began to weaken gradually, and then to undergo distortion—instead of strengthening, the bell caused an obvious weakening of the unconditioned defensive reflex (see Fig. 3d). Particularly indicative in this respect were those combinations in which the conditioned stimulus, applied against the background of the unconditioned reflex, was discontinued a few seconds before the cessation of action of the current: after stopping the bell, the unconditioned motor reaction often was again intensified (tests 46n3, 49n3). As in the experiments considered above (see Fig. 3b) the inhibitory effects of the bell also developed before this stimulus had completely lost its ability to induce a conditioned reaction when applied in isolation (see Fig. 3d; test 9). Complete inhibition of the temporary connection took place only after 51 coverings. We subsequently restored

the conditioned reflex to the bell by renewing its ordinary reinforcements with the electric current. When applied against a background of unconditioned stimulation, the bell was once more capable of causing intensification of the motor defensive reaction of the dog. We obtained analogous results in experiments on the dogs Sil'va and Ryzhaya.

The pattern of development of inhibition during covering of a conditioned reflex induced in the usual manner was thus similar to the pattern of extinction of the temporary connection formed by covering with intensification. This is evidence of a common mechanism of the phenomena under investigation.

We consider that the results described in this paper, taken as a whole, confirm our previous hypothesis of the role of the gradient of unconditioned stimulation in the the mechanism of formation and consolidation of a temporary nervous connection [7, 9]. A temporary connection arises and is consolidated only if the action of the conditioned stimulus is combined with the unconditioned stimulus at the moment of its appearance or sudden intensification. A connection is not formed or, if it is formed, it is inevitably inhibited as soon as the action of the conditioned stimulus ceases to be reinforced by the appearance or the intensification of the unconditioned stimulation.

SUMMARY

Positive conditioned reflexes may be elaborated not only be the usual combination of stimuli, but also if the indifferent agent is applied repeatedly against the background of preexisting unconditioned electrical skin stimulation; each repeated application of this agent was accompanied by intensification of the acting electric current. Differentiation may be formed to the positive "reflex to intensification" if another stimulus, also used against the background of an unconditioned reflex, is not accompanied by intensified current. In this case the differentiating agent begins to exercise a considerable inhibitory effect upon the unconditioned defense reflex. Analogous effects were also observed after repeated applications of the usual conditioned stimulus against the background of an unconditioned reflex. This leads to the conclusion that a combination of the action of a conditioned reflex with a change in the strength of unconditioned stimulation is of decisive significance for the formation and reinforcement of the already elaborated association.

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THE CONSTANCY OF CONDITIONED RESPIRATORY REFLEXES AND ITS USE AS AN INDEX OF THE CORTICAL DYNAMICS IN MAN

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Many authors have drawn attention to the inconstancy of the various forms of conditioned reflexes in man and animals. Descriptions have been given, for instance, of the periodic disappearance of an induced motor reflex [12], and of the inconstancy of the salivary [9], respiratory [3, 4, 10], speech-motor [5], and other reflexes in man.

It should be mentioned that this disappearance of the reflexes in the investigations by the authors cited above was not associated with the action of any relevant factors causing the external or internal inhibition of these conditioned reflexes. We consider that the fact that such a "spontaneous" inhibition of the conditioned reflexes may occur merits special attention and analysis.

In this communication we describe the results of an investigation of the dynamics of the conditioned respiratory reflexes of the healthy adult human subject.

EXPERIMENTAL METHOD

The investigations were carried out on 34 athletes. Three forms of conditioned respiratory reflexes were studied: to a direct stimulus (ammonia vapor), to vocal reinforcement (the words "hold the breath"), and to mental reproduction of muscular work, actually carried out a minute before the investigation. A more detailed account of the technique of the investigation has been given earlier [1]. The first two types of conditioned reflexes were expressed by holding the breath, and the third by an increase in the rate of respiration. The conditioned and differential stimuli consisted of different tones from a sound generator. The use of positive and inhibiting signals was not in the form of a stereotype. The respiratory reactions were recorded pneumographically

EXPERIMENTAL RESULTS

Conditioned reflexes to the direct stimulus were formed in all the subjects, up to 20 combinations being required at most for this purpose, and their final consolidation required a further 3 to 50 combinations. Conditioned reflexes to vocal reinforcement were formed in 96% of persons, and to mental reproduction of muscular work, in 78%. The formation of the last two types of conditioned reflexes required not more than ten combinations in the great majority of subjects. These reflexes were consolidated at the same time as they were formed. It must be pointed out that there is no single criterion in the literature of the consolidation of a conditioned reflex [6, 7, 8, 11, 13]. We considered that the conditioned reflex was consolidated if it appeared in five successive tests.

After the above-mentioned types of respiratory conditioned reflexes had been consolidated, for the next few days we studied the pattern of their appearance. It was found that conditioned respiratory reflexes appear constantly in only a few persons; in the overwhelming majority of subjects inhibition of the conditioned reflexes is observed in occasional tests, and in some, even throughout occasional days of the experiment. Under these circumstances absence of reflexes is not caused by the manifestation of any form of inhibition (external or internal) that could be accounted for.

Special attention was directed to the development of the conditioned reflex in the first test on each day of the experiment. In a group of subjects, for instance, it was inhibited in each experiment, in others, for the first 3-6 experiments. In a small proportion of subjects the reflex to the first test was always well marked (see Table).

It is apparent from the table that at the beginning of the experiment the reflex to mental reproduction of muscular work was subjected to the greatest degree of inhibition, and that which was most resistant was the reflex to vocal reinforcement. It must be stressed that restoration of the conditioned respiratory reflex is possible in some cases also without reinforcement by an unconditioned or vocal stimulus, but only after the repeated application of the isolated conditioned signal.

In a, b, c (Fig. 1; athletes V.D. and V.G.) are shown the conditioned respiratory reflexes to the mental reproduction of muscular work, in the form of a change in the frequency and amplitude of respiration, and also the absence of a respiratory reaction to the application of an inhibiting stimulus. The cuts from the pneumograms

Manifestation of Conditioned Respiratory Reflexes in the First Tests (as % of Total Number of Cases)

Presence of conditioned reflex	Conditioned reflex		
	to direct stimulus	to vocal reinforcement	to mental reproduction of muscular work
Reflex always present initially in experiment	27.3	60.0	16.2
Reflex inconstantly present initially	57.6	16.7	33.3
Reflex always absent initially	15.1	23.3	50.5

indicate the beginning of the individual experiments. When the positive tone was discontinued (T-100+), in the first tests inhibition of the conditioned reflex was visible; a respiratory reaction appeared to the vocal reinforcement "Repeat mentally the exercises with the dumbbells" (RMED). In repetitions of the tests, the conditioned reflex was well marked. In c (athlete V.D.) inhibition of an already consolidated conditioned reflex is shown in the first test, formed in response to the vocal reinforcement "hold the breath". In the second test there was a well-marked conditioned reflex to the isolated application of the positive tone (T-2000+) in the form of holding the breath. In d (athlete V.D.) are shown the conditioned reflexes to the smell of ammonia. Inhibition of the conditioned reflex may be seen on withdrawal in the first test, and a well-marked reflex is present in the second test.

The inhibition of the conditioned reflex in the first test of the experiment may be regarded as a diminution of the ability to concentrate the process of excitation in time, which to some degree characterizes the strength of this process. The fact that inhibition of the conditioned reflex takes place initially in the experiment may therefore be used as an index for evaluation of the process of excitation.

The ratio between the number of tests in which the conditioned reflex was marked and the total of applications of the conditioned signal in this particular series of experiments, expressed as a percentage, was designated by us the index of constancy of the conditioned reflexes – the "total number of manifestations of the conditioned reflex in percent." In the calculations those tests were excluded in which a manifestation of inhibition that could be accounted for (for example: external, successive,

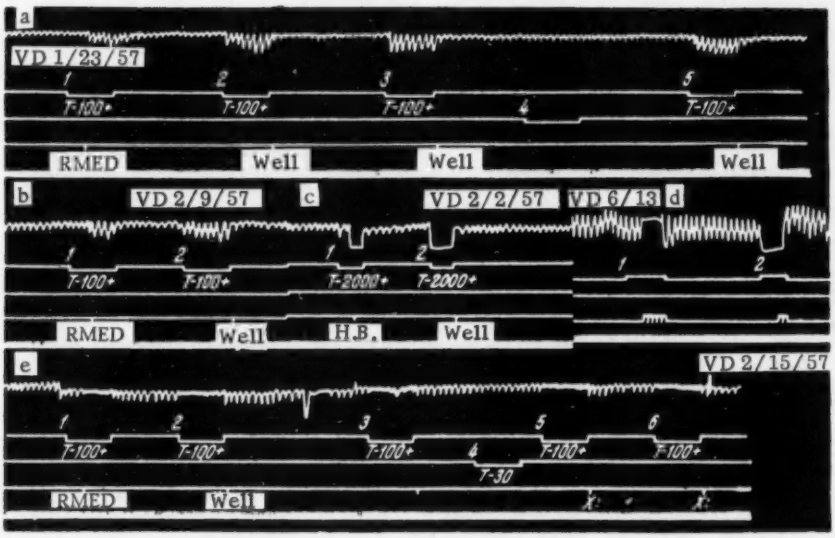

Fig. 1. Inhibition of the conditioned reflex initially in the experiment. Significance of the curves (from above,down): pneumogram; marker of action of positive conditioned stimulus; marker of action of differential stimulus; marker of reinforcement (speech or smell); time marker.

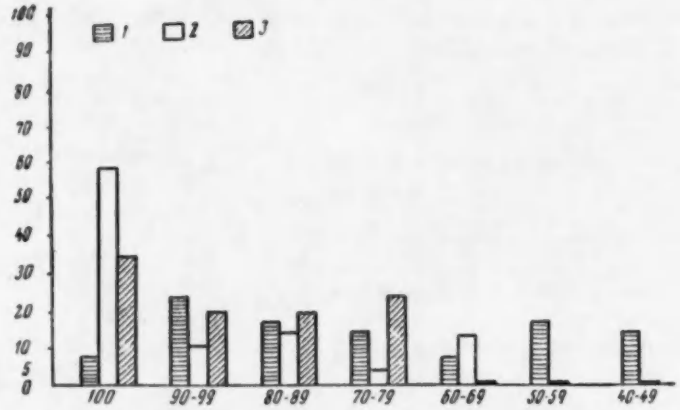

Fig. 2. Constancy of manifestation of conditioned respiratory reflexes. Along the axis of ordinates – number of persons (in %) in whom the index of "total number of manifestations of conditioned reflex in %" reached the value indicated in figures along the axis of abscissae. 1) Conditioned reflex to direct stimulus (ammonia); 2) conditioned reflex to vocal reinforcement; 3) conditioned reflex to mental reproduction of muscular work.

and so on) could be detected. These findings are shown in Fig. 2. We can see that absolute constancy of appearance of the conditioned reflex (100%) is observed most frequently in respect to reflexes formed to vocal reinforcement and next to those formed to mental reproduction of muscular work, i.e., to stimuli addressed to the second signal system. The conditioned respiratory reflexes to the direct stimulus (ammonia) were distinguished by the least constancy of manifestation. Under these circumstances a 100% manifestation of the conditioned reflex was observed in individual subjects only. In the case of reflexes to stimulation of the second signal system, the total percentage of manifestation was not less than 60, whereas in the case of reflexes to stimulation of the first signal system such a low index was observed in one third of all subjects.

It may thus be inferred that conditioned respiratory reflexes may be inhibited without the participation of any agents that are accountable for.

We regard this fact as the result of a constant struggle between the processes of excitation and inhibition, as takes place in the period of consolidation of the conditioned reflex. Analysis of the results obtained leads to the supposition that directly after the first period of consolidation of the reflex a "second period of consolidation" [2] may be seen, when the conditioned reflex is especially prone to inhibition from the second signal system. At this time inhibition of the conditioned reflexes may be observed not only in individual tests, but also in individual experiments. These phenomena have been described by other authors [4, 9, 11], who claim that the mechanism of this inhibition is associated with foci of excitation in the second signal system. T.V. Pleshkova [11], for instance, who examined the character of manifestation of the conditoned blinking reflex from the moment of "first appearance" to the state of "final consolidation", states that there were periods when the reflexes either were diminished or disappeared completely. This was attributed by the author to inhibition, external in its mechanism, from the second signal system. We fully share this point of view and consider that this greater proneness of the conditioned reflexes to inhibition exists only at a particular period of their formation. The duration and character of this period differ in different individuals.

The inhibition described is reversible in character, as is confirmed by the possibility of restoration of the conditioned reflexes by the application of conditioned signals even without reinforcement, and also by the creation of definite conditions of investigation in which inhibition from the second signal system is diminished as was described by V.A. Novi [9].

Having passed through the "second period of consolidation", when interaction between the processes of excitation and inhibition from both signal systems takes place, the conditioned reflex is consolidated to such an extent that in some persons it may even become inert. In some cases inhibition from the second signal system is preponderant, and the reflex may finally be inhibited even after it has been in existence a long time.

The total percentage manifestation of the conditioned reflex thus reflects the interaction between the fundamental nervous processes of excitation and inhibition, and also the neurodynamics during different functional states in man, and it may be used to define the higher nervous activity in man. Inconstancy in the

manifestation of the conditoned reflexes may be regarded as a physiological phenomenon reflecting the changing relationships between the internal and external environments of the subject under investigation.

SUMMARY

The author investigated the constancy of manifestation of the three types of conditioned respiratory reflexes elaborated in response to stimuli addressed to the first and second signalling systems in healthy adult athletes, i.e., to ammonia vapor, to speech reinforcement, and to mental reproduction of muscular work. The general percentage of conditioned reflex manifestation was determined in relation to the total number of positive signals. Inconstancy of conditioned reflex manifestation was regarded as a physiological phenomenon reflecting the dynamics of the between the external and internal environment of the subject to inhibition, evidently originating from the second signalling system. Inhibition of conditioned reflexes is noted during the first test on the day of experiment, lasting for a longer or shorter period in the course of the investigation; this is assessed as a decreased ability of the excitation process to be concentrated in time.

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THE MECHANISMS OF CHEMORECEPTION

Communication I. Relationship Between the Acetylcholine Concentration and the Reflex Production in Cholinergic Receptors

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The elucidation of the mechanism of chemoreception is of practical and theoretical interest. The literature on this subject comprises a considerable number of papers. Many aspects of the physiology of chemoreception are still, however, inadequately studied. Very little attention has been paid to the quantitative aspects of the relationship between the chemical agent and the chemoreceptors; in particular there is no information on the relationship between the acetylcholine concentration and the reflexes arising from cholinergic receptors

Acetylcholine, being a powerful stimulator of chemoreceptors, evidently has a general reflex influence in addition to a local action at the moment of its secretion.

It has been shown [4, 8] that the effectiveness of the action of acetylcholine on the cholinergic receptors of heart muscle is dependent on the acetylcholine concentration. These investigation, however, were carried out on cold-blooded animals (frogs). Moreover, these authors were not concerned with reflexes from cholinergic receptors. Meanwhile,the work of V.N. Chernigovskii and his coworkers and of other authors has shown convincingly that reflexes from the chemoreceptors of any organ have an action on the function of nearly every organ and system of the body.

Acetylcholine has two quite opposite effects on the vasomotor system. The pressor action of acetylcholine, for instance, when applied to the chemoreceptors of normal organs isolated from the circulation of the blood, is well known. Conversely, when acetylcholine penetrates into the general circulation of the blood [3] or is injected intravenously [1], it has a depressor effect. In either case a connection is observed between the concentration of acetylcholine and the degree of intensify of the vascular reaction. Attention to this was first drawn by V.N. Chernigovskii, who carried out experiments to study the relationship between the reflex response and the strength of the interoceptove stimulation [7].

The importance of a detailed explanation of this relationship is perfectly clear; however, we found no other reports of research into this problem in the literature and we decided to make a more detailed study of these aspects.

EXPERIMENTAL METHOD

One of the indices of the function of the chemoreceptors is the reflex from them on any organ. As an indicator of the activity of the cholinergic receptors, we therefore selected the reflex on the arterial pressure. Investigation of the reflexes from the chemoreceptors was carried out in acute experiments on 20 cats under urethane anesthesia, by the well-known method of V.N. Chernigovskii [6]. The depth of the anesthesia was adjusted to give disappearance of the corneal reflex. A segment of small intestine 20 cm in length, isolated from the circulation of the blood but preserving its nerve connections, was perfused with oxygenated Tyrode solution at a temperature of 37-39°. For the investigation we chose a caudal segment of the ileum. The arterial pressure was recorded on a chymograph with a mercury manometer, connected to the carotid artery. Solutions of acetylcholine were prepared in the same Tyrode solution in concentrations of: 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8} g/ml. The cholinergic receptors were stimulated by 1 ml of acetylcholine solution of required concentration, injected into the flow of perfusion fluid next to the arterial cannula. The magnitude of the reflex was calculated from the level of the arterial pressure before application of the stimulus. The order of use of the different concentrations was deliberately varied.

EXPERIMENTAL RESULTS

In the course of the investigations a clear relationship was observed between the changes in the arterial pressure level in response to stimulation of the cholinergic receptors with different concentrations of acetylcholine.

In one of the experiments, for example, stimulation of the receptors with acetylcholine solution in a dilution of 10^{-3} caused an increase in the arterial pressure of 44 mm Hg; a solution in a concentration of 10^{-4} raised the arterial pressure by 32 mm Hg; the pressor effect from a solution in a concentration of 10^{-5} was 18 mm Hg, 10^{-6} – 10 mm and 10^{-7} – only 4 mm; a concentration of 10^{-8} had hardly any effect on the arterial presssure, i.e., it was the threshold value.

These experimental results indicate that the magnitude of the reflex is directly dependent on the acetylcholine concentration. The disproportion noted in individual cases between the acetylcholine concentration and the reflex which it caused could be entirely attributed to possible experimental error: The acetylcholine solution, when injected into the flow of perfusion fluid, underwent further dilution, but not always to the same degree.

Not all the animals showed the same sensitivity to acetylcholine. The threshold concentration of acetylcholine for some animals was 10^{-7}, for others 10^{-6}, and in a few, 10^{-5}. The higher concentrations behaved in a similar way. A concentration of 10^{-3} in one animal caued an increase of 56 mm Hg in the arterial pressure, whereas in some animals this concentration increased the arterial pressure by only 20 mm Hg. In all cases, however, the magnitude of the reflexes was more or less directly dependent on the concentration of the stimulus.

From the results of experiments on 20 animals we obtained the following mean values of the magnitude of the reflexes for each concentration of acetylcholine:

Acetylcholine concentration (in g/ml)

10^{-3} 10^{-4} 10^{-5} 10^{-6} 10^{-7} 10^{-8}

Respective magnitude of reflex rise in arterial pressure (in mm Hg)

31.6 17.2 7.7 4.0 2.0 0

These results are shown graphically in Figs. 1 and 2. The graph of the reflexes in Fig. 1 is in the form of a parabola, and the logarithmic graph (see Fig. 2) is in the form of a straight line, which indicates a direct, but not directly proportional, relationship between the value of the reflex and the acetylcholine concentration. This relationship may be expressed by the formula:

$$H_2 = H_1 \cdot 2^{\lg \frac{C_2}{C_1}},$$

where C_1 is the first concentration of acetylcholine; C_2, the second concentration of the drug; H_1, the magnitude of the reflex in response to C_1; and H_2, that in response to C_2.

It may be concluded from these investigations that the intensity of the reflexes from the cholinergic receptors of the vessels of the small intestine in the cat corresponds strictly to the concentration of the stimulus

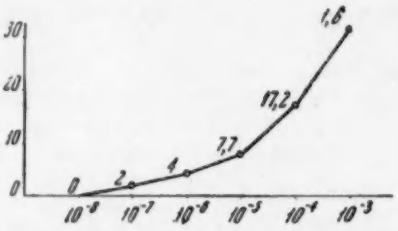

Fig. 1. Relationship of magnitude of reflex to acetylcholine concentration. On abscissae: concentration of acetylcholine (in grams/ml); on ordinates: magnitude of reflex (in mm Hg).

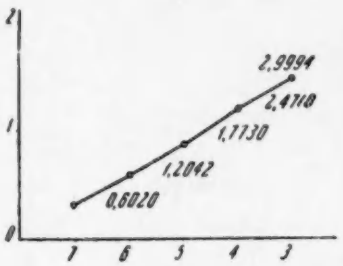

Fig. 2. Logarithmic graph of the magnitudes presented in Fig. 1.

Deviations from this relationship do not exceed 3-7%, and may be attributed to the margin of experimental error.

The quantitative relationships between the acetylcholine concentration and the intensity of the reflexes from the receptors are distinctive in character. From the existing work [7] it might have been expected that a twofold change in the magnitude of the reflex would require a twofold change in the acetylcholine concentration, which could be expressed by the formula:

$$H_2 = \frac{C_2 H_1}{C_1}.$$

Our results showed, however, that a twofold change in the intensity of the reflex called for a tenfold change in the acetylcholine concentration (see the figures cited above), as expressed by the formula which we deduced. If this finding is a regular feature for any chemical compound, it merits particular attention.

As a result of the experiments it was found that an increase in the acetylcholine concentration from 10^{-3} to 10^{-2} was not accompanied by a twofold increase in the intensity of the reflex. The reflex was increased, but not twofold – in fact, much less. This phenomenon was due either to the fact that an acetylcholine concentration of 10^{-3} constitutes almost a full load for the cholinergic receptors, or to the fact that the reaction of the vasomotor system to stimulation of these receptors by

a concentration of 10^{-9} has reached almost its maximum limit.

Since the threshold concentration of acetylcholine for the intestinal vessels of the cat is on the average 10^{-8} g/ml (1 γ%), it is obvious that such a concentration of acetylcholine is already present in these vessels, reaching them as a result of the continuous functioning of tissue structures. The application of a solution in this concentration to the cholinergic receptors does not, therefore, cause any change in the existing concentration of acetylcholine in the vessels or in the function of the receptors. Hence it may be accepted that the normal concentration of acetylcholine in the vessels of the small intestine is 1 γ%; in indivual cases it may vary between 0.1 and 10.0 γ%. In this respect the results of our experiments are reasonably close to the data in the literature concerning the acetylcholine content of the rabbit's blood [2], namely 10^{-9}-10^{-8} g/ml, and the acetylcholine content of the rectus femoris muscle [5] of the adult rabbit (0.12 γ/g muscle).

SUMMARY

The author has established a direct, but not proportional relationship between the concentration of acetylcholine and the intensity of the reflexes from cholinergic receptors expressed by the formula:

$$H_2 = H_1 \cdot 2^{\lg \frac{C_2}{C_1}}$$

where C_1 is the first and C_2 – the second concentration of acetylcholine; H_1 – the value of the reflex in response to C_2 and H_2 – in response to C_2.

The threshold concentration of acetylcholine cholinergic receptors of the small intestine is 10^{-8} g/ml. Hence the author considers the acetylcholine content in the small intestine vessels of cats to average normally 1α%.

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NATURE AND ORIGIN OF AUTOMATIC ACTIVITY IN THE RESPIRATORY CENTER OF THE FROG

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An analysis of information concerning the activity of the respiratory center [2, 4-9, 11, 16, 18-20] indicates that in spite of more than 200 years of study there is still indecision concerning the cardinal problem of the structure of the respiratory center and the localization and origin of its automatic activity. This is explained, apparently, by the complexity of structure and physiological connections, particularly in higher animals. An investigation of the comparative physiological aspects of the problem may assist clarification of the origin of automatic activity in this region.

In connection with this problem, we investigated the functional changes which originate in different parts of the respiratory center of frogs after alteration of automatic respiratory center excitation. Frogs were selected for study because they are one of the first animals to possess a pulmonary respiratory apparatus with a specific mechanism regulating its activity. It was therefore possible to study the natute and origin of automatic activity of the respiratory center in a simplified system whose original schema was unobscured by auxiliary connections.

In the investigations of Ariens-Kappers [17], Bernard [12], and Herrick [15] it was established that impulses from the respiratory muscles and superficial mucosa of the respiratory organ of frogs pass through the VII, IX and X sensory nerves to the respiratory center. The greater part of the fibers involved are directed caudally, forming the fasciculus solitarius (isolated tract). The nucleus fasciculi solitarii is also located in the region where these nerves enter the brainstem. Herrick called this nucleus the "strategic locus" of the nervous system with regard to visceral function. In 1947 A.I. Roitbak noted: "It is extremely tempting to assume that the nucleus of the respiratory bundle is precisely the automatic center. Most of the axons from cells of this nucleus are directed to the motor nuclei of the IX and X pairs of nerves, crossing part of the network of the formatio reticularis". In 1957, Baumgarten [13] also suggested that the nucleus fasciculi solitarii is the respiratory center. Chemical or faradic stimulation at the entrance of the X nerve into the medulla oblongata of frogs with arrested respiration provokes excitation of the respiratory center, whereas stimulation of other parts of the brain under these conditions does not produce this result [5, 6, etc.]. Experiments by B.D. Kravchinskii [3] showed that it is possible to switch the automatic activity on and off, since it is the input from receptors in the aortic zone which is the factor determining the activity of the respiratory center in frogs.

METHOD

Experiments were conducted from 1956-1959 on Rana esculenta and Rana ridibunda, using animals with strong pulmonary respiration. Before each experiment the medulla oblongata of the animal was exposed. In order to study activity of the "inspiration" center (or of its efferent part), a preparation was made of the geniohyoid muscle; for study of the "expiration" center, a preparation of the external oblique muscle of the trunk was used. These muscles, respectively, are related to inspiration and expiration. Subsequent observations were made of contraction of these muscles following localized, juxtalimenal stimulation of their corresponding regions in the medulla. The frogs were fastened, spine upwards, on a plate with an opening opposite the heart and muscles of the fundus of the oral cavity. The reference electrode (silver plate 0.5 cm^2) was fastened to cranial bone. A nichrome microelectrode with glass insulation was used for stimulation. The diameter of the microelectrode tip was 30μ. Location of the electrode tip was primarily determined by the response of corresponding inspiration or expiration muscles to threshold stimulation. After the experiment additional histological verification of the location of the tip of the stimulating electrode was made (hematoxylin-stained microscopic sections). At the beginning of the experiment, the functional characteristics of stimulation of a brainstem region duing automatic activity were determined for the following: 1) Onset of expiration (for inspiratory muscles); 2) onset of inspiration (for expiratory muscles). Subsequently, a small piece of cotton wool, soaked with a 2% solution

of novocain, was applied to the region of bifurcation of the aorta, and the same parameter was measured every 3 minutes during the resulting cessation of respiration. Preliminary experiments indicated that placing a small piece of cotton, soaked with novocain, on the lymphatic sac does not produce cessation of respiration. Consequently, cessation of respiration in the first case was produced only as a result of blocking the input from aortic receptors, and not as a consequence of the action of novocain on the respiratory center.

RESULTS

It was found that the geniohyoid muscle (as well as other inspiratory muscles) may contract even during stimulation of related fibers of neurons located at the level of the entrance of the vagus nerve bundle. It was also found that during stimulation of the brain at the level of outflow of the II cerebrospinal root, which according to Ecker [14] corresponds to the XII pair of cranial nerves of warm-blooded animals, contraction of only the geniohyoid muscle may occur. Histological examination indicated that in the first case the tip of the stimulating electrode was located among cell bodies near the fasciculus solitarius (Fig. 1), and that in the second case the electrode tip was in a region of motor elements (ventral horn). In the latter case the fasciculus solitarius

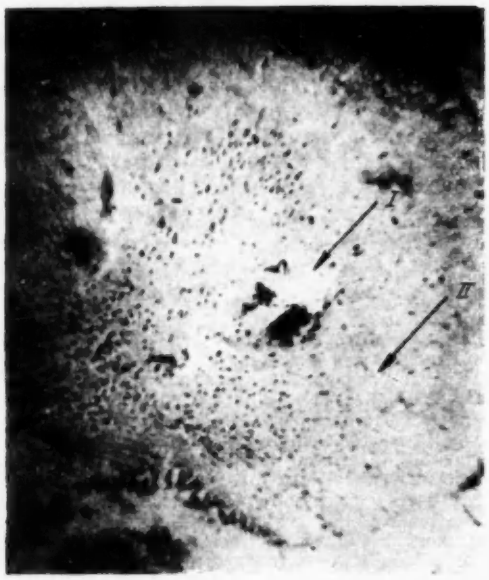

Fig. 1. Medulla oblongata of frog (experiment of March 26, 1959). Section at level of entry of IX and X pairs of cranial nerves. Region of the inspiration center. Microphotograph taken with a 9 × 12 photographic attachment. Ocular 4 x, objective 12 x. Hematoxylin stain. I) Location of tip of stimulating electrode. II) Fasciculus solitarius.

Fig. 2. Medulla oblongata of frog. Microscopic section at level immediately above entry of II cerebrospinal root (dorsal part). Region of the expiration center. Photography, magnification, stains and marks as indicated in Fig. 1.

was found closer to the dorsal part of the medulla (see lower part of Fig. 3). Consequently, motor neurons of the geniohyoid muscle make connection with units situated at the level of entry of fibers of the X pair of nerves. Stimulation of the indicated region causes contraction of all inspiratory muscles. Efferent nerve cells (motorneurons) of these muscles are partially located at the same level (anterior and posterior deglutitory muscles, innervated by the vagus), and partially at the level of entry of the II cerebrospinal root (e.g., geniohyoid muscle). These characteristics were used in a special investigation of the functional characteristics of the efferent part of the respiratory center.

The nerve elements of expiratory muscles are caudally disposed, and just above the level of entry of the II cerebrospinal roots. Stimulation of this region caused contraction of expiratory muscles, particularly the external oblique muscle of the trunk. Histological examination indicated that the tip of the microelectrode during responses of this type was located in the dorsal part of the medulla near the fasciculus solitarius (Fig. 2).

The motor neurons of expiratory muscles are located at the level of entry of the III-VIII pairs of cranial nerves. Thus the histological controls in conjuction with local stimulation indicated the presence of two centers in the medulla of the frog, located near the fasciculus solitarius and connected with excitation of inspiratory and expiratory muscles. This is consistent with previous studies of the respiratory center of warm-blooded animals by a series of Russian and foreign authors (cf. references cited in first paragraph).

The existence, however, of these centers does not solve the problem of the origin of their excitation. In connection with this, by means of novocain block of aortic zone input, we measured physiological parameters of the indicated regions during cessation of the automatic activity of the respiratory center. In experiment No. 3 (March 26, 1959) measurements were made in the inspiration region. The location of the tip of the stimulating microelectrode is illustrated in Fig. 1. Immediately prior to the effect of novocain, the rheobase was 11 v, chronaxie 0.37σ, and accomodation constant (λ) 183σ. These values were changed as soon as 1.5 minutes after the onset of the novocain effect. At 15 minutes the rheobase was 19 v, chronaxie 2.22σ, accomodation constant (λ) 77σ. Similar changes in excitability, accomodation, and chronaxie were observed in the other experiments. Mean values for 25 experiments are given in Table 1. The mean rheobase was increased 2 times, chronaxie was lengthened 4-10 times, and the accomodation constant (λ) was sharply reduced. Measurement of parameters in the expiration region and in the efferent part of the respiratory center revealed no abrupt changes even throughout the first hour following cessation of impulses from the aortic zone. Thus, for example, in experiment No. 2 (March 13, 1959) the rheobase in the efferent region immediately prior to novocain block was 7 v, chronaxie 0.18σ, and accomodation constant (λ) 100σ. Following novacaine block the rheobase was 8.5 v, chronaxie 0.2σ, and accomodation constant (λ) 113σ. The location of the tip of the electrode is illustrated in Fig. 3. Mean values for 25 experiments are given in Table 1.

In the experiment of June 1, 1959, the rheobase in the expiration region prior to the action of novocain was 8 v, chronaxie 0.19σ, accomodation constant (γ) 333σ. Subsequently, the rheobase was 9.5 v, chronaxie 0.19σ, accomodation constant (γ) 375σ. Location of the microelectrode tip in this experiment is shown in Fig. 2. Mean values for 25 experiments are given in Table 1.

From all the above observations it can be concluded that automatic excitation originates in the region containing motor neurons of inspiratory muscles. The region containing motor neurons of expiratory muscles is not affected by cessation of impulses from the aorta. Under natural conditions the expiration center of the frog is excited secondarily: either by means of its reciprocal relations with the inspiration center, or, as indicated by Woldring and Dirken [19] for warm-blooded animals, as a result of its activation by receptors of the lung and proprioceptors of respiratory muscles, with the activity of these being synchronized with excitation in the inspiration center.

Of great interest for the general physiology of the nervous system is the fact that with the background of automatic activity the functional characteristics of the inspiration center are approximated by both its own efferent part and the expiration center. After exclusion of the input from the aortic zone, the characteristics of the inspiration center abruptly change, whereas in

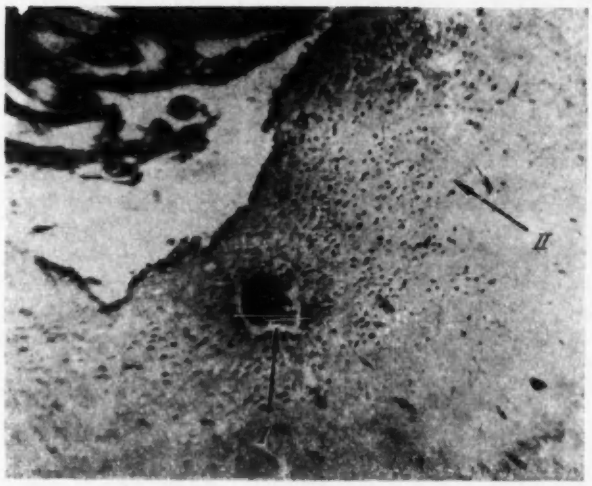

Fig. 3. Medulla oblongata of frog. Section at level of entry of II pair of cerebrospinal roots (ventral part). Efferent region of inspiration center. Magnification, stain, and marks same as Figs. 1 and 2.

Physiological Parameters of Various Regions of the Respiratory Center of the Frog with and without Automatic Activity (mean values for 25 experiments in each series)

Region of respiratory center	With background of automatic activity			During absence of automatic activity		
	rheobase (in v)	chronaxie (in σ)	accomodation constant (λ)	rheobase (in v)	chronaxie (in σ)	accomodation constant (λ)
Inspiration	8.5	0.381	153.4	16.7	3.301	110.9
Expiration	8.43	0.19	288.2	7.35	0.19	288.9
Efferent	9.03	0.338	296.8	9.4	0.386	302

other regions of the respiratory center, as mentioned above, changes are not observed. Therefore, during steady input from the aortic receptors, the inspiration region is functionally equivalent to a motor region, and it is known that motor regions are highly unstable. This instability, according to A.A. Ukhtomskii [10], can produce functional instability of afferent input, as a result of assimilation of the rhythm of the neural center. Actually, in the inspiration center, input exclusion caused an abrupt reduction of excitability, an increase in chronaxie, and a decrease in accomodation time, i.e., complex functional changes characteristic of the state of parabiosis. This agrees with the idea of N.V. Golikov [1] that "elimination of the supportive excitation of a region of low lability causes the development in it of a functional parabiosis. This occurs regardless of the absence of recurrent stimulation, and is precisely due to elimination of the instability-producing excitation".

SUMMARY

Stimulation of interneurons at the level of entrance of the vagus fasciculus near the fasciculus solitarius provokes local contraction of only "inspiratory" muscles, while stimulation of the interneurons at the level of the exit of the II spinal root, also near the dorsal portion, causes local contraction of only "expiratory" muscles. In normal automatic activity the functional characteristics of the "inspiratory" and "expiratory centers" is identical with that of the efferent region. With the exclusion of the impulsation from the aortic receptors, when automatism ceases, the rheobase in the "inspiratory center" assumes a double value, while chronaxie is increased 4–10 times, accomodation constant (λ) is cut nearly in half. At the same time the functional characteristics of both the "expiratory" center and the efferent area approach the initial one. Hence a conclusion was drawn that the automatic excitation is being formed only in the region of the "inspiratory center".

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CHANGES IN THE CARDIAC OUTPUT AND GENERAL PERIPHERAL RESISTANCE AFTER OBSTRUCTION OF A CORONARY ARTERY

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Our national literature contains no published papers regarding the changes in the cardiac output and general peripheral resistance after obstruction of a coronary artery. With the help of a Russian electromagnetic rotameter (type RÉD, model 3103-A) and an apparatus for cardiac catheterization, we made a continuous recording of cardiac output and mean arterial pressure.

EXPERIMENTAL METHODS

Using an animal under intravenous thiopental sodium anesthesia after the preliminary administration of morphine hydrochloride in a dose of 5 mg per 1 kg of weight, we made a longitudinal sternal section (switching the animal to artificial respiration at the requisite moment) to expose the thorax and dissected out the brachiocephalic, left subclavian and both carotid arteries and the aorta, distal to the origin of the left subclavian artery. The femoral artery was also dissected out. Then we exposed the pericardial cavity and, using 2-4 ml of a 2% novocain solution, dissected out the circumflex or descending branch of the left coronary artery.

Next, heparin was intravenously injected in a dose of 5 mg per 1 kg of weight, and the rotameter was attached to the animal as depicted in the diagram, Fig. 1. The plastic tubes were used to connect the aortic end of the left subclavian artery 1 to the inlet opening of the rotameter 2. The proximal section of the femoral artery 3 and the cephalic sections of the carotid arteries 4, 5 were connected with the outlet of the rotameter 6.

When the cannulation had been completed, we opened the clamps on the left subclavian a and carotid b arteries. After the blood flow from the subclavian artery through the rotameter to the carotid arteries was established, the brachiocephalic artery was ligated 7. Then a clamp was immediately placed on the aorta distal to the origin of the left subclavian artery 8, and, simultaneously, the clamp on the femoral artery c was opened. From this time on, the blood from the aorta passed through the left subclavian artery into the rota-

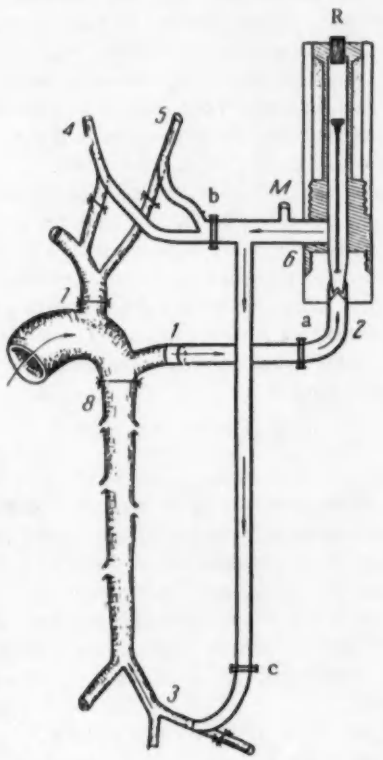


Fig. 1. Diagram showing how rotameter was connected with animal. 1) Aortic end of left subclavian artery; 2) rotameter inlet; 3) proximal end of femoral artery; 4,5) cephalic ends of carotid arteries; 6) rotameter outlet; 7) brachiocephalic artery; 8) place of aortic obstruction [a clamp at rotameter inlet; b) clamp for tubes to carotid arteries; c) clamp on tube carrying blood to femoral artery; R) rotameter; M) electromagnetic manometer.

meter and, from the latter, went to the carotid and femoral arteries. This provided continuous measurement by the rotameter of the total volume of blood expelled by the left ventricle into the aorta, excluding the blood flowing out from the aorta into the coronary arteries.

Three to five minutes after constriction of the aorta, when a constant value had been established for the blood flow through the rotameter, we began to record the curves of the volumetric blood flow and the mean blood pressure on a POB-14 oscillograph. A Moskite* clamp was applied at the mouths of the dissected-out branches of the left coronary artery during the continuous recording of these curves. After developing the photographic record, we studied the changes which developed in the volumetric blood flow and the mean blood pressure.

It is evident from Green's studies [3] that the correlation between the height of the pressure and the volume of the blood flow in the artery is in the form of a parabola which curves toward the axis of the pressure. High pressure in the tubes causes extra stimulation of the myocardium due to the increase it causes in the volume of the coronary blood flow. The diameter of the plastic tubes, therefore, has to be such that the mean arterial pressure inside them is 80-100 mm of mercury with a normal animal. The external appearance of the coronary arteries is a good index of how correctly this question has been decided. The coronary arteries should look the same before and after cannulation of the large arteries. Any visible distention of the coronary arteries with blood due to a passive increase in their diameter indicates that the mean arterial pressure in the tube system is not correct.

EXPERIMENTAL RESULTS

Thirty-six experiments, ten of which were preparatory, were performed by the method described. Oscillograms from 19 experiments were used to analyze the hemodynamic disturbances (see table). In four experiments (19, 21, 22 and 23), we reproduced successive obstruction of the circumflex and descending branches of the left coronary artery. The observations are brief due to the early development of ventricular fibrillation.

In 18 out of the 19 experiments, obstruction of the coronary artery was attended by a decrease in the output of the left ventricle; this decrease either developed gradually, becoming clearly evident after the first 20-60 seconds, or developed acutely immediately after the application of the clamp (Fig. 2).

In six experiments, the cardiac output remained constantly reduced during the entire observation period. In 12 experiments, it rose to the original level at different intervals following the obstruction, either from time to time (in 10 experiments) or stably (in one experiment). After an initial decrease, the cardiac output in experiment 24 not only regained, but at times even exceeded by 8.6% the maximal value of the original period; this evidently should be regarded as a manifestation of hypercompensation of the heart's contractile function.

In four experiments, we attempted to produce a stabler and more pronounced decrease in the output by increasing the mass of ischemic tissue. Obstruction of the second branch of the left coronary artery was attended by a more acute decrease in the output, but ventricular fibrillation developed in three experiments soon after the application of the second clamp. The results of the longer lasting experiment 22 showed that the capacity of the unaffected myocardium to maintain the output at the former level was not exceeded even after obstruction of the mouths of both branches of the left coronary artery.

The changes in the mean blood pressure generally reflected the changes in output. In 11 experiments (3, 7, 8, 10, 12, 15, 17, 19, 21, 22 and 24), the blood pressure and output decreased simultaneously. In seven experiments, the blood pressure remained somewhat lowered, but, in ten experiments, increased briefly or stably to the original level. In experiment 18, the mild decrease in output which occurred after obstruction of the coronary artery was not attended by any fall of the blood pressure, which toward the end of the experiment had become even higher than during the control period. The variations in output and blood pressure observed in experiment 14 were no greater than the variations observed in these values during the control period.

In spite of the brevity of the observations, one can assume that obstruction of a coronary artery under these

* Transliteration of Russian — Publisher.

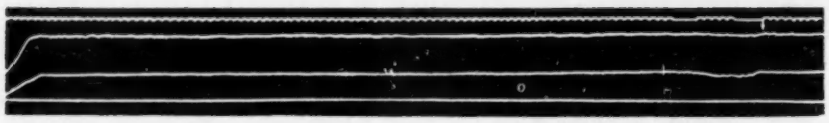


Fig. 2. Change in output and mean blood pressure after obstructure (↓) of circumflex branch of left coronary artery. Experiment 20 (August 22, 1958). The output decreased from 22.8-22 to 18.5 ml/sec after obstruction of the artery. The blood pressure fell from 92 to 87 mm after 3 seconds and then to 85 mm of mercury after 40 seconds. Curves (from top to bottom) show: time in seconds; mean blood pressure; output of left ventricle; zero line. Paper moving from right to left.

Changes in the Hemodynamic Indices after Obstruction of the Coronary Artery

Experiment	Initial deviations						Deviations during experiment														Duration of observation after obstruction
	cardiac output		mean arterial pressure		general peripheral resistance		cardiac output					mean arterial pressure				general peripheral resistance					
							restoration to original level					increase to original level				increase					
	decreases	no changes	fall	no changes	increase (in % of maximal original level)	no changes	stable	periodic	periodic increase over orig. level	stable decrease	no conclusive fluctuations	stable	periodic	periodic increase over orig. level	stable fall	no conclusive fluctuations	constant	with periodic decreases: to original level	with periodic decreases: below original level	no changes	
3	+		+		121,2					+			+					+			1min. 17sec.
5	+		+		259,7			+					+						+		0,9 sec.
6	+		+		123,4					+					+				+		33min.
7	+		+		110,4			+				+						+			4 min. 36 sec.
8	+		+		—	+				+					+			+			57sec.
10	+		+		103,6					+					+				+		2 min. 48 sec.
12	+		+		108,5			+					+					+			2 min.
13	+		+		101,8			+							+			+			1 min. 22 sec.
14	—	+	—	+		+					+					+				+	1 min. 18 sec.
15	+		+		—	+		+					+							+	60min. 05 sec.
17	+		+		107,6			+					+					+			16min.
18	+		+	+	101,8		+							+				+			34 min.
19a	+		+		106,3			+					+					+			} 30 min. 15 sec.
19б	+		+		113,7 *					+					+			+			
20	+		+		110,2					+					+		+				2 min. 54 sec.
21a	+		+		116,1			+				+						+			} 26 min.
21b	+		+		135,7 *					+			+					+			
22a	+		+		103,4			+					+					+			} 75 min.
22b	+		+		128,7 *			+					+					+			
22c	+		+		134,6 *			+					+					+			
23a	+		+		106,7			+							+			+			} 5min. 03 sec.
23b	+		+		1 32,9*					+					+					+	
24	+		+		115,3				+				+					+			1 min. 30 sec.
26	+		+		111,5					+					+			+			2min. 33 sec.

*After the double obstruction, the general peripheral resistance was computed in relation to its value before application of the clamp.

specific experimental conditions causes a temporary decrease in the cardiac output and the mean blood pressure.

The deviations of the experimental hemodynamic indices from the original values were variable, even diametrically opposite to each other in some experiments. To explain the role of the cardiac and vascular factors in the hemodynamic disturbances which developed, the general peripheral resistance was determined according to the formula:

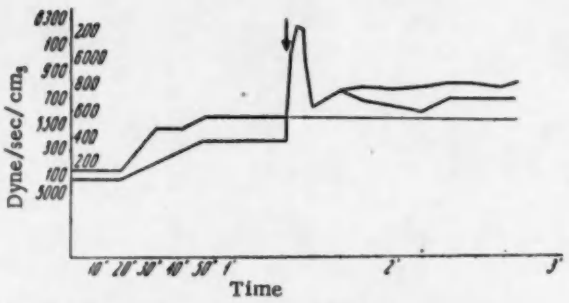
$$GPR = \frac{Mm \cdot 1332}{O},$$

where GPR is the general peripheral resistance in absolute units, Mm is the mean arterial pressure in millimeters of mercury and O is the cardiac output in milliliters per second; 1332 is the coefficient by means of which, according to one of the accepted international systems for measuring physical values (C.G.S. system), units of resistance are converted into absolute units (dyne · sec/cm^5

Immediately after obstruction of the coronary artery, the general peripheral resistance increased markedly in 16 experiments, indicating an attendant increase of the vasomotor tonus in the systemic circulation. As one of the authors of this article has reported in [1], the venous supply to the heart remains ample in experimental myocardial infarction. On this basis, it would seem that the decreased output observed in our experiments was due to primary affection of the contractile function of the heart. This is also suggested by the prior decrease of the output observed in several experiments.

Despite the sometimes clearly apparent fall of the mean arterial pressure, the general peripheral resistance was observed to increase in all 16 experiments. Therefore, the fall of arterial pressure was not due to lowered vascular tonus or to any insufficiency of the venous supply, but was, in the final analysis, a hemodyamic manifestation of primary affection of the contractile function of the heart. The hemodynamic disorders observed would seem to be unique in character, as this type of disorder does not occur in shock or paresis of the vasomotor tonus.

Peripheral resistance to the blood flow increased after the obstruction, but in only one experiment did it remain increased throughout the observation period (Fig. 3). In 15 experiments, it quickly returned to the original level. However, no stable restoration of the original correlations between pressure and output was observed. In 12 experiments, the peripheral resistance again increased and remained so, although it periodically decreased to the original level. In experiment 8, the general peripheral resistance initially remained within the original range, but increased somewhat toward the end. No conclusive fluctuations in the peripheral resistance were observed in experiment 14.



↓ Obstruction of circumflex branch of left coronary artery

Fig. 3. Increase in the general peripheral resistance after obstruction of the circumflex branch of the left coronary artery. Experiment 20 (August 8, 1959).

Therefore, we observed a stable or periodic increase in the general peripheral resistance in 17 out of 19 experiments. The increase of the vasomotor tonus in the systemic circulation is evidently as typical a consequence of coronary artery obstruction as is the decrease in output. The four experiments with successive obstruction of the circumflex and descending branches of the left coronary artery provided especially convincing confirmation of this conclusion. Each time, obstruction of the large branch was attended by a sharper increase in the general peripheral resistance.

There was no apparent change in the general peripheral resistance in three experiments, which indicates that an increase in vasomotor tonus is evidently not a universal, although an extremely frequent, reaction of the vascular system to obstruction of a coronary artery. Our continuous registration of the hemodynamic indices showed other indications of the complex nature of the changes observed in the vascular tonus. In two experiments (5 and 23) on a background of sharply increasing peripheral resistance, there were brief periods (lasting 1 and 6 seconds) in which it fell considerably below the original level. The single decreases of the peripheral resistance observed in experiments 6 and 10 were less conclusive.

Our experimental methods permit only a summary evaluation of the vasomotor changes in the systemic circulation. The increased general peripheral resistance observed in the majority of cases could be due to a predomination of vasoconstrictor processes, possibly in only certain vascular regions, over vasodilatation or unchanged vascular tonus in other regions of the organism. The antipodal fluctuations of the general peripheral resistance, which sometimes lasted several seconds, can only be caused by the interaction of conflicting trends of vasomotor changes with a brief predomination of the vasodilatation processes, probably in certain vascular regions, over the vasoconstrictor processes in others.

In the literature available to us, we found only two works studying the regional of the vascular tonus in experimental myocardial infarction. According to M.N.

Levy and A.L. Frankel in [4], obstruction of a coronary artery is always attended by an increase in the resistance to the blood flow in a dog's posterior extremity. Bing and co-workers [2], in one series of experiments, observed decreased resistance in the coronary circulation, evidently indicating active dilatation of the coronary arteries.

Comparison of our experimental results with the literary data allows us to hypothesize that obstruction of a coronary artery is attended by complex reorganization of the tonus in different vascular regions of the systemic circulation, owing to which redistribution of the reduced cardiac output is effected. The humoral and reflex mechanisms of this specific reaction of the vascular system to an acutely decreased output, which reaction is also observed in other pathological conditions on models of myocardial infarction, have not yet received sufficient study.

Therefore, the acute decrease in output following obstruction of a coronary artery is attended by a peculiar reorganization of the vascular tonus in the systemic circulation. The rather regular increase in the general peripheral resistance observed in the course of most of the experiments indicates a predomination of the vasoconstrictor processes over the vasodilatation processes. The complex mechanism of the increase in general peripheral resistance helps to redistribute more economically the reduced output, i.e., acts as an adaptational reaction, providing for the maintenance of a more or less stable level of arterial pressure, on the one hand, and for the sustenance of the contractile properties of the unaffected myocardium on the other by increasing the venous supply to the heart and maintaining the maximal possible coronary circulation volume.

SUMMARY

The left subclavicular, both carotid and femoral arteries were shunted; then the brachiocephalic artery and aorta were obstructed, distal to the origin of the left subclavicular artery. The output of the left ventricle, excluding that portion of it which flows into the coronary arteries, entered the rotameter through the left subclavicular artery and flowed into the distal sections of carotid arteries and the proximal sections of the femoral artery. The average blood pressure in the system was measured by an electric manometer. From these data, general peripheral resistance could be estimated according to the data obtained. An average blood pressure of 80–100 mm Hg is the best at which to study the changes of hemodynamic indices occurring after obstruction of the coronary artery.

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CHANGES IN THE INTENSITY OF PROTEIN SYNTHESIS IN THE MYOCARDIUM DURING COMPENSATORY HYPERFUNCTION OF THE HEART

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Compensatory hyperfunction of the heart is one of the chief symptoms of circulatory pathology. In heart defects, hypertonic disease and hypertonia of the pulmonary circulation, the maintenance of normal hemodynamic indices, clinical compensation and, therefore, the patient's life largely depends on stable compensatory hyperfunction of the heart. Under these conditions, the heart works continuously under an increased load for many years without reaching a state of exhaustion. The mechanism which prevents cardiac exhaustion, despite the continuous nature of its hyperfunction, has not yet been explained; the existing theory of the "reserve forces of the heart" needs rather than offers explanation. Also unexplained is the mechanism of the cardiac exhaustion which often sets in after many years of compensatory hyperfunction and marks the beginning of decompensation.

The myocardial hypertrophy which develops in all cases of compensatory hyperfunction of the heart is very important in these processes.

In this work, we studied the intensity of protein synthesis in the myocardium of animals with compensatory hyperfunction and hypertrophy of the heart, induced by an experimental defect.

EXPERIMENTAL METHODS

The intensity of protein synthesis in the heart muscle was estimated according to the inclusion in the proteins in vivo of the amino acid methionine, labeled with S^{35}. Radiomethionine was injected subcutaneously into rabbits in a dose of 600 impulses per minute per 1 g of animal weight. In order to standardize the conditions, all the rabbits were starved for 18 hours before the administration of the methionine. The rabbits were killed four hours after the injection, i.e., during the period of approximately maximal inclusion of the amino acid in the proteins, and the radioactivity of the heart and blood plasma was determined. The heart was washed free of blood, ground into a homogeneous state and treated three times with 20 and 5% trichloroacetic acid, then with alcohol, a mixture of alcohol and ether, and, finally, with ether to obtain preparative precipitation of the dry protein. An end-window counter was used to determine the radioactivity of each batch of this protein (10 mg).

Experimental aortic stenosis was induced in the experimental animals by an earlier described method [2, 3]. The hearts of these animals were examined for radioactivity 2, 7, 30 and 155 days after the creation of the heart defect. Four experimental and four control animals were examined at each of these intervals.

EXPERIMENTAL RESULTS

The results obtained in all the experiments are given in the table.

The data in the table show that, during the emergency stage, when the heart is rapidly increasing in weight, there is also an increase of about 100% in the intensity of methionine inclusion in the proteins of the myocardium. The intensity of this inclusion then gradually diminished, reaching the normal level after a month, when the stage of relatively stable myocardial hyperfunction is observed, and the weight of the heart has become stabilized. Five months after the creation of the defect, methionine inclusion had decreased to approximately half the normal level.

There was some increase in the intensity of methionine inclusion in the plasma proteins during the first seven days following the creation of the defect. On the whole, however, the data in the table indicate a lack of proportionality between the intensity of methionine inclusion in the myocardial proteins and that in the plasma proteins. For example, five months after the creation of the defect, the intensity of methionine inclusion in the proteins of the heart was sharply reduced, but, in the plasma proteins, it was normal or even increased. In our experiments, therefore, the intensity of methionine inclusion in the myocardial proteins was not by any means determined by the content of methionine in the plasma proteins, but depended on

the dynamics of the metabolic processes in the heart muscle.

According to contemporary opinion, the intensity of radiomethionine inclusion reflects the protein synthesis rate, or to be more exact, the rate at which the protein structures of the myocardium are renewed. Therefore, the data obtained give reason to suppose that the vigorous development of hypertrophy which takes place during the emergency stage is attended by a corresponding increase in the protein synthesis rate in the myocardium; during the stage of relatively stable hyperfunction, normalization of the synthesis rate is observed, while during the stage of gradual exhaustion and cardiosclerosis, the rate of synthesis decreases, sharply inhibiting the normal restoration process of the myocardial protein structures.

Intensity of S^{35} Methionine Inclusion in the Proteins of the Heart and Blood Plasma of Rabbits with Experimental Heart Defect

Rabbit No.	Time after operation (in days)	Number of impulses per min per 10 mg of protein			
		heart		plasma	
		absolute values	average	absolute values	average
6	Control	102	126	182	169
7	"	121		148	
8	"	132		189	
9	"	148		156	
3	2	223	252	185	158
4	2	228		122	
5	2	288		148	
2	2	270		178	
17	7	309	239	348	298
18	7	175		260	
19	7	220		285	
1	7	250		340	
10	30	140	150	—	—
11	30	132		—	
12	30	190			
13	30	140			
14	155	54	71	195	199
15	155	70		179	
16	155	84		212	
20	155	77		210	

The data obtained regarding the intensity of protein synthesis in the myocardium during experimental heart defect, combined with the results published earlier of research conducted by one of us on the same model [2-8], permit the conclusion that the heart passes through three main stages in the process of compensatory hyperfunction.

The First Stage. The emergency stage of acute overstress is characterized by the development of tonogenic cardiac dilatation, which approximately doubles the diameter of the heart. There is a 10-12% daily increase in the weight of the heart. Protein synthesis in the myocardium accelerates to about twice the original rate; the muscle fibers appear swollen, and the myofibrils pull apart from each other; the glycogen content of the myocardium decreases to 1/2-1/3 its former value, the lactic acid content increases 18-20%, the phosphocreatine content is reduced to 1/10 its original value and the ATP content remains normal. Changes in the T wave and the S-T segment are observed on the electrocardiogram.

The Second Stage. During the stage of prolonged and relatively stable hyperfunction, the heart is characterized by stable dimensions. The weight of the heart is also stabilized at about 100% more than the normal weight. The rate of protein synthesis in the myocardium is normal, and there are no signs of protein or fatty dystrophy; hypertrophied muscle fibers with compactly arranged myofibrils are observed. The myocardial content of phosphocreatine, ATP and glycogen is normal, but the lactic acid content is 200% the normal. No changes in the T wave and the S-T segment are apparent on the electrocardiogram, but there is a shift of the electrical axis of the heart to the left.

The Third Stage. This stage of progressive exhaustion is characterized by stable heart weight, some increase in the dimensions of the heart due to additional dilatation and pronounced and progressive cardiosclerosis. Foci of fatty dystrophy appear in the muscle fibers; the protein synthesis rate in the myocardium is 2-2½ times less than the normal rate; the glycogen content of the myocardium is normal, and the lactic acid content is 250-300% of the normal value. The electrocardiograph shows an increasing shift to the left of the electrical axis of the heart.

The transitions between these three stages are gradual. One very important factor in the transition of the heart from the first, or emergency, stage to the second stage of relatively stable hyperfunction [8, 9] is the rapidly developing hypertrophy based on the increase we established in the rate of protein synthesis in the myocardium. The hypertrophy increases the mass of the myocardium, especially the mass of sarcosomes within which are localized the enzyme systems of oxidizing phosphorylation which provide for the aerobic resynthesis of ATP. After the development of hypertrophy, therefore, the disappearance of the signs peculiar to the emergency stage, indicating mobilization of anaerobic sources of ATP resynthesis, is evident from the decrease in the myocardial supply of glycogen and phosphocreatine. Hypertrophy increases the ability of the heart as a whole to resynthesize high-energy compounds, but at the same time causes the concentration of capillaries in the myocardium to decrease to almost half the original [13]. Due to the acute prolongation of the path of oxygen diffusion from the capillary wall to the center of the muscle fiber, a certain degree of myocardial hypoxia develops, which in turn causes an accumulation

of lactic acid in the myocardium. During the first stage of the process, therefore, hypertrophy is a very important factor in the heart's adaptation to a long increased load, but it subsequently becomes the prelude to cardiosclerosis and other pathologic changes in the myocardium.

The inhibition of protein synthesis in the myocardium established in our experiments may be very important to the development of cardiosclerosis and cardiac exhaustion in the final stage of the process.

It has recently been shown that change in the properties of actomyosin, the principal protein contractile complex of the myocardium, becomes of essential importance in the development of insufficiency of a hypertrophied heart under conditions of flaws and hypertonia. In this case, the myosin content of the myocardium is reduced, the viscosity of the actomyosin precipitated in pure form decreases, and the ability of glycerinated muscle fibers to contract upon the addition of the ATP is diminished [10, 12]. One can assume that the disturbance of the ability of the myocardial contractile proteins to convert the potential energy contained in the high-energy phosphoric acid compounds into the kinetic energy of cardiac contractions is connected with the inhibition of the normal restoration process of the myocardial protein structures.

There are two reasons which can be given at this time for the inhibition of protein synthesis in the myocardium under conditions of cardiac hypertrophy.

1. Accumulation of lactic acid in the myocardium regularly accompanies cardiac compensatory hyperfunction of long duration; this indicates oxygen deficiency and a decrease in the level of the oxidation-reduction processes and is attended by some decrease in the ATP content of the myocardium. Disturbances of this type in the energy sources utilized in protein synthesis naturally can result in a reduced rate of protein synthesis and in the inhibition we observed in the exhaustion stage of the normal restoration process of the myocardial protein structures.

2. It is generally acknowledged that myocardial hypertrophy is not attended by hyperplasia of the muscle fibers, but consists only of an increase in their size; consequently, there is a much greater increase in the mass of sarcoplasm in a hypertrophied myocardium than in the mass of nuclei. A relative deficiency of desoxyribonucleic acid DNA, which is chiefly concentrated in the cell nuclei and is of great importance to the process of protein synthesis, can develop in such a situation [11]. Such a relative deficiency of DNA can, in turn, disturb the synthesis of protein in the myocardium.

SUMMARY

Compensatory hyperfunction of the heart in vitium cordis, hypertension and other diseases of the circulatory system connected with the cardiac overstrain of long duration represents the main factor in preservation of normal hemodynamic indices and clinical compensation. The rate of protein synthesis in the myocardium was studied under conditions of compensatory cardiac hyperfunction provoked by experimentally induced vitium cordis. As established, rapid development of hypertrophy during the first emergency stage of compensatory hyperfunction is accompanied by an intensified rate of protein synthesis (it about doubles). In the second stage of relatively stable hyperfunction the rate of protein synthesis is normal, while at the third stage—characterized by cardiosclerosis and progressive exhaustion—it is reduced 2–2.5 times. This depression of the normal renovation process of the protein myocardial structures may bring about a change in the contractile proteins of the heart, thus playing an important role in the development of cardiosclerosis and cardiac insufficiency.

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DESCRIBING THE ELECTROCARDIOGRAPHIC CHANGES IN DOGS WITH EXPERIMENTAL ATHEROSCLEROSIS UNDER CONDITIONS OF LONG OBSERVATION

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The definite ideas recently formulated as to the morphology and morphogenesis of atherosclerosis are chiefly based on the widely known and accepted research of Russian pathologists [1, 2, 4, 5, 8]. These works stress experimental reproduction of atherosclerosis in animals and systematic comparison of the morphological data obtained experimentally with the corresponding changes in man at different stages in the development of the pathologic condition. But, although these works on the problem of atherosclerosis are of great significance, extensive exploitation of the physiological experiment is needed to explain the primary pathogenic mechanisms of its development.

Besides the choice of animal, one must also take into account in reproducing a biological model of atherosclerosis the slow and gradual character of the development of this process in man. The initial insidious stage in the development of atherosclerosis, primarily associated with disturbances in the area of nervous regulations [4, 14], undoubtedly deserves particular attention and requires special experimental methods for its demonstration.

In experiments on dogs, the use of large cholesterol loads combined with strong influences on the metabolic processes (suppression of thyroid gland function) seems to give results which differ greatly from the functional changes which take place in the organism during the natural development of atherosclerosis, especially in the initial stages. In this respect, it would seem that the experimental pathologic condition could be more efficiently created by employing pathologic influences on the organism which are milder, but of longer duration. One can also assume that compensatory changes would be more apparent under conditions of slowly developing pathologic changes, making it possible to study at the same time the dynamics of the functional disorders and the mechanisms of their compensation.

Our work, which was based on the above ideas, consisted of pathologic-anatomical examination of the material after two and three-year systematic functional investigations, performed on the same groups of dogs throughout, during the development of experimental atherosclerosis in the animals.

The influences employed in the work to induce atherosclerotic changes in the dogs' cardiovascular systems were relatively mild.

We were particularly interested in the possibility of comparing the functional changes which occurred in these investigations with the known facts concerning the morphogenesis of atherosclerosis which indicate the variable course of this pathologic condition [1, 2, 4, 5, 8, 14]. It seems rational to assume that the fluctuations and cycles in the morphological picture of atherosclerosis are connected with the changes in the mechanisms which regulate the physiological functions of the organisms and the variations in the metabolic processes at different stages in the development of the pathologic condition.

This article reports some results of electrocardiographic research performed on dogs with experimental atherosclerosis. We were largely concerned with the changes in the cardiac activity of the animals which could be detected with the aid of extra stimulations (intravenous adrenalin injection) at a stage in the experiments when the resting electrocardiogram did not yet show pathologic changes.

EXPERIMENTAL METHODS

The experiments were performed on five dogs aged 5-7 years and weighing 7-10 kg. Methylthiouracil was administered enterally to the dogs daily throughout the investigations in gradually decreasing doses, beginning with 1 g during the first 1-2 months of the experiment and subsequently decreasing to 0.3 g. Chol-

esterol was administered with the food (without being first dissolved in oil) in a daily dose of 2-3 g. Periodically, generally during 10 months of the first 1½ years of observation, the animals each received 30,000-50,000 units daily of vitamin D. An intravenous infusion (into the anteroexternal vein of a posterior extremity) of a 1:10,000 solution of adrenalin in a dose of 0.2-0.6 ml was used as one of the functional tests. The adrenalin administration was accompanied by a continously recorded electrocardiogram (taken with an EKP-5 apparatus in the second standard lead with the dog standing) and was used at least thrice monthly under chamber conditions; the experimental setup was kept strictly constant throughout the two and three-year investigations. We used the electrocardiograms of five intact dogs, recorded on a background of the adrenalin test, as the control.

EXPERIMENTAL RESULTS

With the adrenalin test, the first changes on the electrocardiogram were apparent in individual dogs the 5th-6th and 8th months of the administration of the preparations inducing the development of atherosclerosis in the animals. As the experiment continued, the pathologic reactions of the heart increased in degree and duration, as well as in the speed with which they were manifested after the administration of adrenalin to the animal. These disturbances lasted 1-2 minutes and were distinguished by pronounced polymorphism. Some of the disturbances in myocardial automatism and conductivity which we observed in the experiments with the administration of adrenalin to the dogs are shown in Figs. 1, 2, and 3.

From the data presented, it is evident that the pathologic changes in the electrocardiographic elements occurred on a background of a sometimes extremely pronounced central effect induced by the adrenalin and, therefore, were to a definite degree associated with changes in the experimental animals of the functional condition of the cardiac center of the vagus nerves. The same doses of adrenalin sometimes retarded the rate of the cardiac contractions in the intact dogs, but this retardation was never as great as in the animals with developing experimental atherosclerosis. At first, the pathologic changes in the electrocardiogram observed with the adrenalin test were brief, the curve returning to its original form at the 40th-60th second of the recording. Besides the retardation of the rate of the cardiac contractions observed during the first 10-20 seconds of the recording, the electrocardiogram also showed changes in the form and direction of the T wave and considerable changes in atrioventricular conductivity, usually tending to retard the latter. Soon, single ventricular and blocked atrial contractions, ventricular extrasystoles, or premature blocked atrial contractions began to attend the administration of adrenalin.

At subsequent stages of the investigation, the disturbances of the principal myocardial functions induced

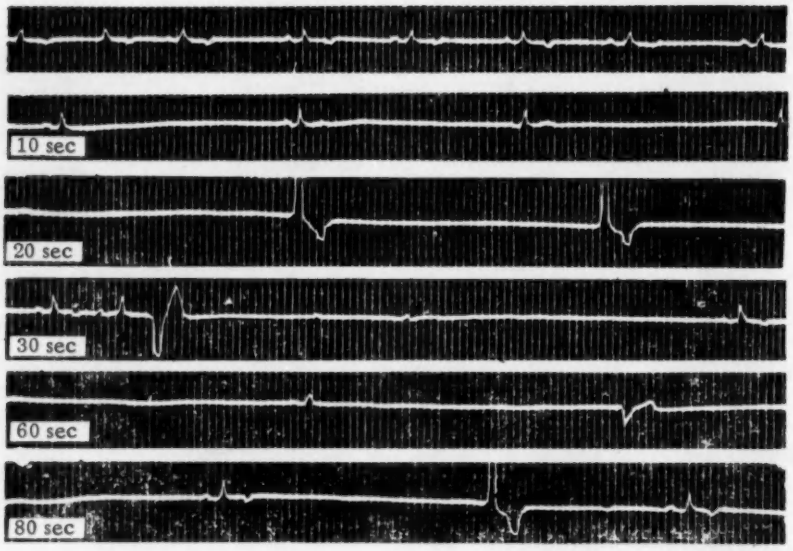

Fig. 1. Electrocardiogram of dog Damka taken April 13, 1957. Top) Initial recording; below) subsequent recordings at different times after the administration of 0.4 ml of the adrenalin solution (1:10,000). Before infusion: RR) 0.55-0.90 sec; PQ) 0.10-0.11 sec; QRS) 0.05-0.06 sec; QT) 0.22-0.23 sec. After infusion: RR) 0.55-2.6 sec; PQ) 0.08-0.15 sec; QRS) 0.06-0.07 sec; QT) 0.21-0.25 sec. Tenth second-change in direction of T wave; starting 20th second-periods of idioventricular rhythm and wandering pacemaker, single ventricular extrasystoles.

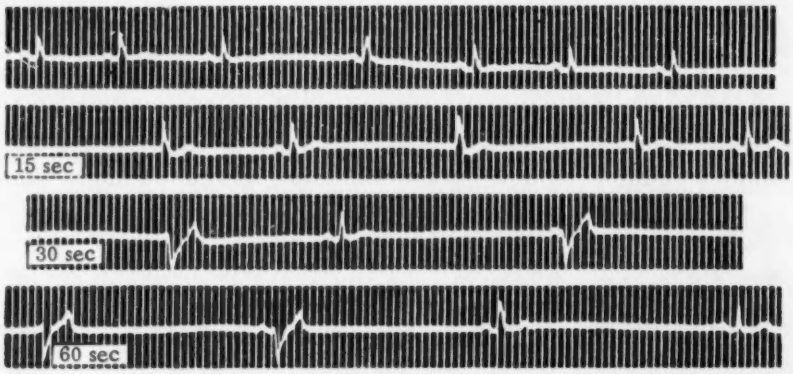

Fig. 2. Electrocardiogram of dog Ryzhka taken January 29, 1957. Top) initial recording, below) subsequent recordings at different times (15, 30 and 60 seconds) after the administration of 0.2 ml of a 1:10,000 adrenalin solution. Fifteenth second: combination of nodal (from middle of atrioventricular node) and sinus rhythms (wandering pacemaker). Subsequently apparent are single ventricular contractions and typical Wolff-Parkinson-White syndrome complexes. PQ) 0.04--0.6 sec; QRS) 0.08 sec.

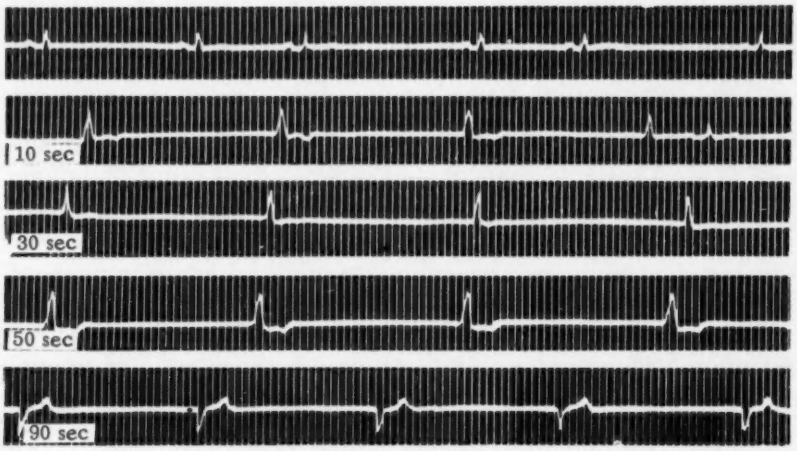

Fig. 3. Portions of electrocardiogram of dog Ryzhka taken March 21, 1957, 10, 30, 50 and 90 seconds after the administration of 0.4 ml of the 1:10,000 adrenalin solution. Successive depression of automatic centers of cardiac excitation with transition to idioventricular rhythm. Pronounced wandering pacemaker. Tenth second: change in direction of T wave. Nodal rhythm with impulses from middle part of atrioventricular node (10th-30th second) combined with intraventricular block. QRS) 0.08 second.

by the adrenalin test became more pronounced and longer lasting, even when the doses of adrenalin were reduced. As well as more pronounced bradycardia, one could observe combinations of complete heart block and ventricular extrasystoles, different types of partial atrioventricular block, periodic coupled rhythm, total depression of the sinus discharges with a gradual dislocation of the focus of stimulation to automatic centers of the II rank (usually nodal rhythm with impulses originating from the middle part of the atrioventricular node), and changeover of the heart to idioventricular rhythm. The wandering pacemaker symptom was extremely pronounced. Particular attention was attracted by the appearance on the electrocardiogram of complexes typical of the Wolff-Parkinson-White syndrome (see Fig. 2), which, in one of the dogs, was often combined with

short periods of paroxysmal tachycardia. As far as is known, this was the first time that this paradoxical disturbance of intraventricular conduction has been clearly observed in classic form under conditions of the development of a pathologic condition in a chronic experiment. The appearance of this electrocardiographic syndrome, the nature of which is still a mystery, was associated with the action of adrenalin on a background of the development in the animals of the given pathologic condition.

In this connection, it is interesting to note that A.V. Lirman [11, 12] observed the Wolff-Parkinson-White syndrome in acute experiments on dogs, stimulating certain sympathetic cardiac ramuli with faradic current. Studying the topography of these ramuli, the author discovered that they take part in the innervation of the atrioventricular system (4th-3rd nerve plexuses and ganglionic areas according to Vorob'ev-Zhuravlev).

Analysis of the electrocardiographic changes described above in the experimental animals under the influence of adrenalin permits the hypothesis that a condition of functional instability of the cardiac conducting system develops in dogs even in the early stages of the atherosclerotic process.

In our experiments, doses of adrenalin which did not cause pathologic changes in the cardiac activity of intact animals, under conditions of the development of atherosclerosis, became extraordinarily stimulating to the organism, even in the initial stages of the experimental condition, causing insidious forms of disturbance of its compensatory mechanisms. These disturbances chiefly affected the intracordal nerve apparatuses and the region of the atrioventricular node in particular, as the Wolff-Parkinson-White phenomenon is, according to experimental data [11, 12, 18], associated with change in the functional condition of the structure elements of the atrioventricular node.

The known facts concerning the considerable change in vascular reactivity, specifically to adrenalin, which occurs in hypercholesteremia and experimental cholesterol atherosclerosis [3, 6, 9, 10, 13, 15, 16, 17] permit the hypothesis that the pathologic changes which develop in the region of the autonomic innervation of the vascular system of the heart disturb the blood supply of various parts of the conducting system by altering their functional condition.

This creates a pathologic background for the extracardial influences (also altered in atherosclerosis) which realize the changes we observed on the electrocardiograms.

The pathologic reactions of the heart to adrenalin in the experiments were striking both for their extraordinary polymorphism and the periodic nature of their manifestation. The electrocardiograms obtained with our systematic use of the adrenalin test throughout the investigations showed the pathologic phenomena to have periods of increase and decrease. In cases of the latter, electrocardiographic disturbances could be induced simply by increasing the dose of adrenalin. In several cases, the functional tests showed a general lack of pathologic changes; specifically, the pathologic changes apparent on the electrocardiogram of the dog Damka taken April 13, 1957 (Fig. 1) were less pronounced on the recordings taken April 27, 1957 and completely absent on May 9 and 27, 1957. Neither was any central adrenalin effect apparent in these instances. The administration of adrenalin to the same dog on May 28, 1957 again induced pathologic changes in the electrocardiogram, which then again gradually disappeared in the subsequent investigations. Towards the end of the 2nd year of observations on this animal, we observed a considerable decrease in the voltage of all the electrocardiogram waves, and the disturbances noted during the functional tests were in most cases confined to variations in the condition of atrioventricular and intraventricular conduction and to changes in the T wave.

The above dynamics of the electrocardiographic indices were observed to varying degrees in all the experimental animals, indicating the fluctuation of the functional condition of the cardiovascular system during the development of experimental atherosclerosis in dogs.

In our investigations, the indices of the lipid-cholesterol metabolism, the blood pressure level, and the condition of the vascular reflexogenic zones [19] also demonstrated a similar picture of phasic functional changes varying in degree and cycles. Comparison of these functional shifts with the data indicating the fluctuating course of the morphological changes in atherosclerosis (also observed on our material [7]) permit one to propose the existence of certain genetic links between them.

SUMMARY

The author presents data concering the dynamics of the ECG changes occurring in dogs following an intravenous injection of adrenalin under conditions of experimental atherosclerosis. As a result of 2- and 3-year systematic investigations, it was established that the adrenalin test aids in detecting latent forms of disturbed physiological compensatory mechanisms, even at the early stages in the development of experimental atherosclerosis. This was manifested in the ECG by various combinations of pathological rhythms of cardiac activity; the negative cardiac shifts were polymorphic, indicating pronounced functional instability of the cardiac conducting system. As noted, the disturbances of the main functions of the cardiac muscle observed in the adrenal tests are cyclic in character with periods of rise and fall in the negative shifts. This is associated with the varying course of morphological changes occurring in atherosclerosis.

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THE EFFECT OF VITAMIN B_{12} ON THE REVERSE DEVELOPMENT OF CHOLESTEROL ATHEROSCLEROSIS IN RABBITS

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Ever since a group of Leningrad pathologists [2,3,7] showed that the reverse development of atherosclerosis was possible in man, many experimental works have been conducted in order to find a way of hastening this process by using substances which prevent the development of experimental atherosclerosis. Since the degree to which experimental atherosclerosis develops depends to a considerable extent on the blood cholesterol level, the substances first tested were those lowering this level.

The works of several authors [6, 7, 9, 11] have demonstrated that thyroidin (dessicated thyroid preparation) has an effect notably accelerating the reverse development of cholesteremia and experimental cholesterol atherosclerosis in rabbits.

When no preparations are used, a period of 6-8 (McMillan, Horlick, Duff) or more (T.A. Sinitsyn) months is required for this process in rabbits and 8-9 months (Horlick and Katz) in chicks, but with the use of thyroidin, the initial signs of reverse development are apparent $1\frac{1}{2}$-$2\frac{1}{2}$ months after the final cholesterol feeding [6].

A combination of thyroidin and estrogens was found to be especially efficient in lowering the cholesterol level and accelerating the reverse development process of atherosclerosis [11]. Heparin, which according to the data of several authors [5, 8, 13], inhibits the development of experimental atherosclerosis, does not significantly affect the reverse development of cholesteremia. The authors differ in their evaluations of heparin's effect on the reverse development of atherosclerosis; acceleration of this process was macroscopically observed by Horlick and Duff in experiments on rabbits, but Akira Horita and Lomis did not observe this phenomenon in experiments on chickens.

Choline's effect on the development of experimental atherosclerosis and the resorption of atheromatous plaques is debatable. It has recently been found that certain vitamins of the B group, particularly vitamin B_{12}, take part in the synthesis of choline and methionine and possibly in the transfer of methyl groups.

At the suggestion of A.L. Myasnikov, Active Member of the AMN SSSR, we studied the effect of vitamin B_{12} on the formation and reverse development of atheromatous elements in the aorta of rabbits, reproducing cholesterol atherosclerosis by the method of Academician N.N. Anichkov. We reported the results of our study investigating vitamin B_{12}'s effect on the development of experimental atherosclerosis in rabbits at the IXth session of the AMN SSSR Institute of Therapy in February, 1958. We demonstrated that the level of cholesterol in the blood did not increase as much in animals which received vitamin B_{12} along with the cholesterol as in the animals given cholesterol alone, so that the phospholipid-cholesterol coefficient figures for the rabbits given vitamin B_{12} were higher, and there was considerably less development of atherosclerosis. Our data were confirmed by the works of Yu.G. Grigorov [4], and Chakravarti and Mukerji [10].

This article presents the results of research on the processes of reverse development of experimental atherosclerosis under the influence of vitamin B_{12}.

EXPERIMENTAL METHODS

The experiments were performed on 24 rabbits of the same breed, sex and age, weighing 1.5-2 kg. Along with their usual food, all the rabbits received a 10% solution of cholesterol in sunflower oil for 105 days in a daily dose of 0.2 g cholesterol per 1 kg of animal weight. We then stopped the cholesterol feeding and divided the rabbits into three groups. The six rabbits of the first group were killed to determine the degree of atherosclerosis development in them at this time; this group served, so to speak, as a control for the other two groups. The remaining 18 rabbits were divided into two groups, identical as to number of animals and blood cholesterol level, forming the second and third groups; these animals were given the usual food ration for 112 days. The nine rabbits of the second group served as the control for the rabbits of the third, or experimental, group, which received a daily subcutaneous injection of 30 μg vitamin B_{12}. Every 25 days throughout the experiment on the

rabbits (217 days), we investigated the cholesterol content of the blood serum according to the Engel'gardt-Smirnova method and determined the lipoid phosphorus. Bloor's method was used to extract the lipoid phosphorus from the blood serum, and the sulfite-hydroquinone method was used for the actual phosphorus determination. The numerical value obtained for the lipoid phosphorus was multiplied by the coefficient 25; the obtained sum of the phosphatides was conditionally assumed to be lecithin, and the lecithin-cholesterol coefficient was computed.

EXPERIMENTAL RESULTS

The level of cholesterol and phospholipids in the blood serum increased considerably in almost all the rabbits during the cholesterol feeding period, and the phospholipid-cholesterol coefficient decreased.

Towards the end of the 105-day cholesterol feeding period, the average cholesterol content of the rabbits' blood serum was 1125 mg% in the first group, 787 mg% in the second, and 740 mg% in the third. The average content of phospholipids was 745 mg% in the first group, 533 mg% in the second group, and 652 mg% in the third. The phospholipid-cholesterol coefficient was 0.65 in the first group, 0.77 in the second, and 0.61 in the third.

During the period of reverse development, the cholesterol and phospholipid levels decreased in both the second, or control, group and the third, or experimental, group.

At the end of the experiment, 112 days after the final cholesterol feeding, the average level of blood cholesterol was 285% in the control group of rabbits, but 142 mg% in the animals which had received vitamin B_{12}; the average content of phospholipids was 191 and 131 mg% respectively, and the phospholipid-cholesterol coefficient was 0.88 and 1.34.

On the basis of the average figures at the end of the experiment, therefore, vitamin B_{12} can be said to have a marked effect on the reverse development of the cholesterol and phospholipid content of the blood (Fig. 1). It should be emphasized, however, that this effect of vitamin B_{12} is not immediately evident.

If the curves of the reverse development of cholesteremia and the phospholipid level, which we constructed from the average levels of these substances during the experiment, are compared, it is evident that the phenomenon of the decrease in the level of blood lipids effected by vitamin B_{12} was not clearly apparent until the 4th month after the final cholesterol feeding. The curves for the first three months were about the same.

The degree to which aortic atherosclerosis had developed was estimated by the weight planimetric method after total staining of the aorta with sudan III; according to this method, the degree to which the aorta is affected by the various atheromatous elements (thick plaques, thin plaques, lipoid spots) is expressed in percent of the

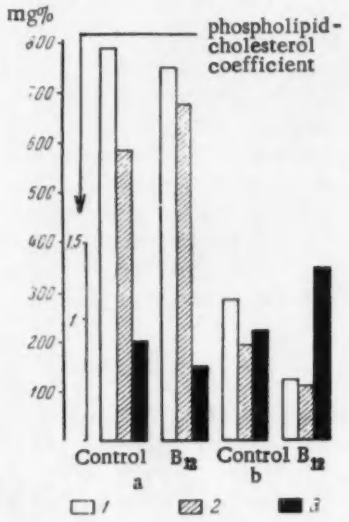

Fig. 1. Average levels of the cholesterol and phospholipid content of the blood and values of the phospholipid-cholesterol coefficient for the rabbits of the second (control and third experimental groups at the end of the cholesterol feeding period and at the end of the experiment. a) At end of cholesterol feeding period; b) at end of experiment; 1) Cholesterol level; 2) phospholipid level; 3) phospholipid-cholesterol coefficient.

total area of the aorta. The sum of all the elements, expressed in percent, is the overall degree of aortic atheromatosis.

The graphs on the left side of Fig. 2 show the degree of aortic atheromatosis in each animal of the three groups, and those on the right show the average values. First of all, one can see from Fig. 2 that the degree of aortic atheromatosis was was considerably higher in the rabbits of the second group, which were fed their customary diet after the end of the cholesterol feeding period, than it was in the rabbits of the first group, which were killed at the end of the 105-day cholesterol feeding period, even though the average blood cholesterol level of the first group at the end of the feeding (1125 mg%) significantly exceeded that of the second group (787 mg%). After the cholesterol feeding was stopped, therefore, intense atherogenesis, which the reverse development processes could not affect in so short a time (112 days), occurred in the rabbits of the second group.

A different picture was observed in the rabbits of the third group, which were given vitamin B_{12} after the cholesterol was discontinued. As Fig. 2 shows, the

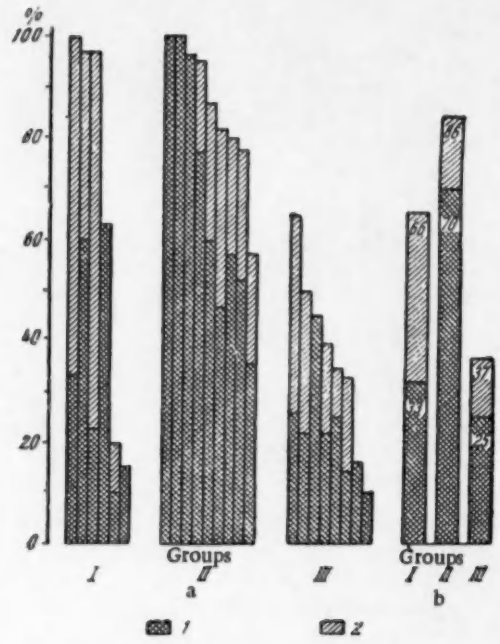

Fig. 2. Degree of aortal atheromatosis. a) Degree of aortal atheromatosis in all three groups; b) average percent of aortal injury in each group; 1) Injury by thick atheromatous plaques; 2) total atheromatosis; I) Rabbits killed after 105-day cholesterol feeding; II) rabbits subsequently fed the usual diet; III) rabbits subsequently given vitamin B_{12}.

degree of aortic atheromatosis in these animals was not only no higher, but definitely lower than in the rabbits of the first group and much lower than the degree of aortic atheromatosis in the second group.

Comparison of the blood lipid level and the degree to which aortic atheromatosis developed in the rabbits of the second and third groups suggests that the considerably lesser degree of aortic atheromatosis observed in the rabbits given vitamin B_{12} cannot be explained solely by the sharper decrease in the blood lipid level of the experimental group as compared with the second group, because, as we have already stressed, this sharper decrease was only clearly apparent towards the end of the experiment. This of course suggested that the reason for the relatively rapid reverse development of aortic atheromatosis observed in the rabbits of the experimental group had to do with the influence of vitamin B_{12} on the process of resorption of lipoid masses from the walls of the aorta. The nine aortas of the rabbits in the second (control) group and the nine aortas of the animals comprising the third (experimental) group were therefore subjected to histological examination. We examined pieces taken from the ascending aorta, the arch of the aorta and the abdominal aorta.

Microscopic examination showed the intima of the aortas from the control rabbits to be considerably thickened by a profuse deposit of fatty substances in the form of large and small drops of fat, which stained red with scarlet red. A large number of fat drops were found in the round cells with well-stained nuclei. In places, fat drops were beginning to pass into the tunica media of the aorta. Staining with picrofuchsin showed a significant number of delicate collagen fibrils in the places of the fat deposits. In the intima of the aortas from the rabbits of the experimental group, which had received vitamin B_{12}, the fat deposits were considerably smaller, and staining with picrofuchsin demonstrated a greater number of collagen fibrils than in the control rabbits. This suggests that the reverse development process of atheromatous plaques was more intense in the experimental rabbits.

SUMMARY

Prolonged vitamin B_{12} administration promotes reverse development of atheromatous elements in the rabbit aorta, affected by cholesterol atherosclerosis. Evidently, accelerated reverse development of cholesterol atherosclerosis is connected with the action of vitamin B_{12} upon the concentration of the lipids in the blood serum.

One cannot exclude, however, the direct activating effect of the vitamin upon the vascular wall elements on which depends the resorption and removal of lipids infiltrating its intima.

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A STUDY OF HIGHER NERVOUS ACTIVITY IN EXPERIMENTAL TUBERCULOSIS

Communication I. Conditioned Food-Secretory Reflexes in Dogs on a Background of Tuberculosis Infection and during Streptomycin Therapy

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(Presented by V.N. Chernigovskii, Active Member of the AMN SSSR)
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Many clinical and clinicophysiological observations have established that disturbances of higher nervous ac activity develop in tuberculosis. There are also data indicating that these disturbances play an important pathogenic role in the development of the tuberculous infection.

It should, however, be stressed that many aspects of this problem remain unexplained. Specifically, although there are several works [1, 2, 3] investigating the influence of the tuberculous infection on the higher nervous activity of experimental animals, there are many sides of this question which remain unexplored (the comparative resistance of positive and negative conditioned reflexes to tuberculosis, the significance of the type of conditioned reflex, the reversibility of the changes which develop in higher nervous activity, etc.).

This article presents results obtained in a study of conditioned food secretory reflexes in dogs infected with tuberculosis during the diseased period, during the treatment of the experimental animals, and at the end of the course of treatment.

EXPERIMENTAL METHODS

The conditioned reflexes were investigated in a soundproof chamber by the food-secretory method. A stereotype of conditioned reflexes was developed in the dogs to the following stimuli: a bell, a light, a positive tone, a gurgle, a negative tone and a bell. The experiments were performed on six dogs, two of which (Chilka and Oka) served as a control (the dogs were infected with tuberculosis, but we did not investigate their conditioned reflexes or administer treatment to them). A total of 247 experiments were performed on four dogs. All the dogs were mongrels. Orsha, Seraya and Moroshka had nervous processes of average strength, were even-tempered and rather lively; Pyatnistyi belonged to the strong, inert type, The infection was produced intravenously with a culture of the Valle strain in a dose of 0.5 mg/kg (Orsha, Moroshka and Chilka) or 0.75 mg/kg (Seraya, Pyatnistyi and Oka). Streptomycin was injected intramuscularly in a dose of 20,000 units per 1 kg of weight, 30-60 minutes after the work with the dogs in the chamber ceased.

EXPERIMENTAL RESULTS

As soon as the second day after the infection, the sum of the positive conditioned reflexes of the dogs of the first group was observed to decrease 70-75% of the original level (Fig. 1). Unconditioned secretion began to decrease significantly towards the end of the first week following infection (by 50-64%), although in one dog (Moroshka), its decrease began at the same time as the decrease in the conditioned (Fig. 1 and 2).

The conditioned secretion to the weaker stimuli decreased before that to the stronger. Increasing sequential inhibition was observed. Two weeks after infection, the total amounts of the dog Orsha's conditioned reflexes had decreased an average of 85% of the original level, in some experiments equalling zero. Unconditioned secretion continued decreased. During this period, one could observe the delayed development or even the complete lack of the conditioned reflex motor food reaction. In several experiments, the dogs fell asleep between the different stimuli. Slight dis-

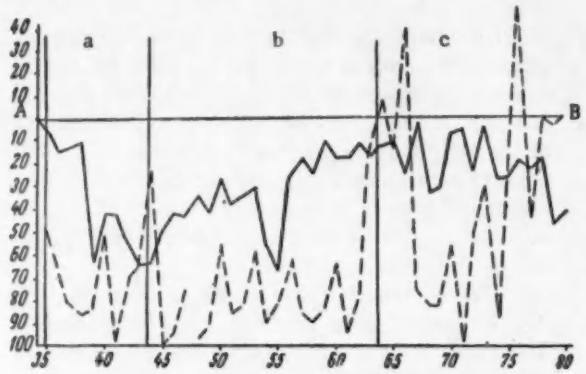

Fig. 1. Relative changes (in percent) in amount of conditioned and unconditioned secretion in the dog Orsha during tuberculosis. Axis of abscissa) number of experiments; axis of ordinate) percent: a) Before streptomycin treatment; b) on background of treatment; c)after treatment ended; A-B) Average initial level of amount of conditioned (- - -) and unconditioned (——) secretion before infection.

inhibition of the differentiation was observed in both dogs in a few experiments.

Streptomycin treatment of the dogs in this group was started the third week after infection. During the first 3-3½ weeks of treatment, conditioned reflex salivation remained at the same low level and,in several experiments on one of the dogs, decreased even further than before the treatment. Except in a few experiments, the differentiation was total. The amount of unconditioned secretion began to increase slightly on the 17th-18th day (Orsha) and at the end of the treatment had almost regained the original (before infection) figures. In the second dog (Moroshka), restoration of the unconditioned secretion began somewhat later and proceeded parallel with restoration of the conditioned.

According to the conditioned reflex indices, relative normalization of the condition of higher nervous activity occurred later than the improvement in the dogs' clinical condition; it was not observed in Orsha until the 50th day or, in Moroshka,until the 40th day of treatment. At this time, the amount of Orsha's conditioned secretion equalled 81%, the amount of Moroshka's,75% (of the original, taken to be 100%); in one experiment, the amount even exceeded the average original level. During this period, the antibiotic injections were discontinued. The tendency of the amounts of the conditioned reflexes to level off continued after the treatment was stopped, but they still did not equal the original level. One of the dogs, Moroshka, died accidentally seven days after the last streptomycin injection. The second dog, Orsha, was sacrificed a rather long time after the treatment was stopped. The autopsy performed on Moroshka showed a mildly expressed tuberculous process—colorless miliary tubercules in every lobe of the lungs and single tubercules in the kidneys and spleen, while the autopsy on Orsha showed only single miliary tubercules in the lungs.

The dose of the tuberculous culture administered to the second group of dogs was 1½ times stronger in order to induce a more serious process. As early as the end of the first week following infection, we observed in the dog Seraya that the sum of the positive reflexes decreased an average of 35%; some disinhibition of the differentiation (1-2 scale divisions; Fig. 3) was also observed. At this point, unconditioned secretion had decreased an average of 8-10%. At the end of the 2nd week (and thereafter), we observed a sharp decrease in conditioned reflex salivation, sometimes coming to (in a few experiments) a total lack of reflex to the weaker stimuli. Sequential inhibition became more intense. The conditioned reflex motor food reactions to the same stimuli were also absent. In addition, there was a sharp decrease (68-70%) in the amount of unconditioned reflex salivation. Unconditioned secretion and the sum of the positive conditioned reflexes then continued to remain at a low level. The dog stopped eating the meat-sugar powder.

During the first eight days of the infection, the sum of the dog Pyatnistyi's positive conditioned reflexes decreased an average of 55%; no disturbance of the differentiation was observed. The decrease in the amount of saliva representing unconditioned food secretion was much greater in the case of Pyatnistyi than in the case of Seraya, reaching 95% of the original level by the 9th day of the disease. The dog began to refuse food as early as the first week after infection; the conditioned secretory and the conditioned motor food reactions "split" (the first was greatly diminished

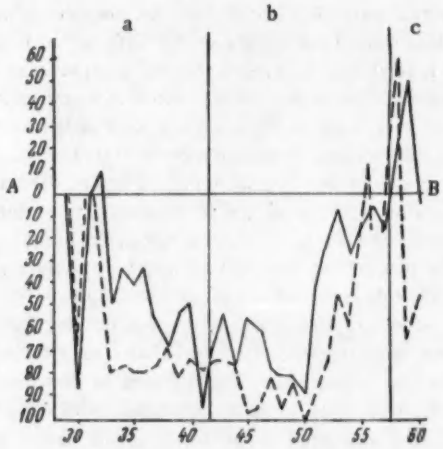

Fig. 2. Relative changes (in percent) in amount of conditioned (- - -) and unconditioned (——) secretion in the dog Moroshka during tuberculosis. Symbols the same as in Fig. 1.

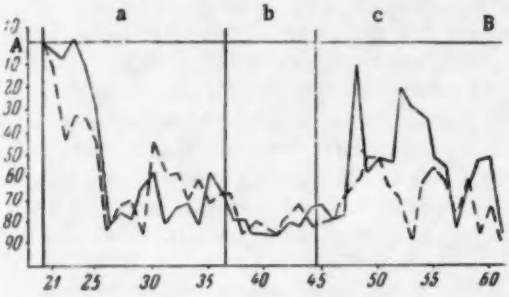

Fig. 3. Relative changes (in percent) in amount of conditioned (- - -) and unconditioned (——) secretion in the dog Seraya during tuberculosis. Symbols the same as in Fig. 1.

while the second was active). Progressive deterioration of the dog's general condition was observed the 3rd week of the infection; the animal lost weight and became weaker. The differentiation was noticeably disinhibited. At this time, the level of conditioned reflex secretion was 75-80% lower than the original. Sequential inhibition had increased.

We began to treat the dog Pyatnistyi the 33rd day of the disease, and the dog Seraya, the 42nd day. The course of treatment lasted 3½ weeks. Throughout the course of treatment, the conditioned and unconditioned reflex saliva secretion of the dog Seraya continued to remain at a low level; conditioned salivation to the strong stimulus (bell) decreased, and the conditioned reflexes to the weak stimuli were particularly inhibited. During the streptomycin treatment of the dog, we noted progressive disturbance of its motor coordination and an ataxic gait. In view of this, the streptomycin injections were discontinued on the 25th day of treatment. The indices of conditioned reflex secretion were low. A tendency to restoration was noted in a few experiments in relation to the unconditioned reflex secretion, but as the animal's condition deteriorated, it too again fell to 85% of the original level. The dog died 4½ months after infection and 68 days after treatment ceased. The autopsy disclosed cachexia, serous exudate in the pleural and pericardial cavities and numerous miliary tubercules in the lungs, liver, spleen and kidneys.

After streptomycin treatment of the dog Pyatnistyi began, conditioned and unconditioned reflex activity continued to decrease. The differentiation was stably disinhibited. Considerable deterioration of the dog's condition was noted about the 30th-35th day after infection: the dog lost weight progressively and died, showing symptoms of increasing weakness. The autopsy disclosed serosanguineous exudate in the pleural and abdominal cavities, many miliary tubercules in the lungs and a solitary focus in the spleen the size of a hazelnut.

The control dogs (Chilka and Oka) were also infected with culture of Koch's bacillus (with 0.5 and 0.75 mg/kg respectively); they were each sacrificed and subjected to pathologico-anatomical examination on the day that we began treatment of their related group of experimental animals. We found a microfocal tuberculous process in the lungs and kidneys of both dogs. Miliary foci were also observed in Chilka's spleen and in Oka's liver.

The data obtained from the autopsies on the control dogs indicated that the treatment of the experimental animals was started on a background of developed experimental tuberculosis in both experimental groups. The experimental results have shown that considerable functional disturbances of the sections of the cerebral cortex participating in the formation and realization of conditioned food secretory reflexes occurred in the experimental dogs under the influence of the tuberculosis infection. This was very clearly indicated by the decrease in the value of the conditioned positive salivary food reflexes. Intensified sequential inhibition was also regularly observed in all the experimental animals.

Another phenomenon observed was disinhibition of the differentiation. This phenomenon, however, was less regular in character. In the first place, it usually developed later than the decrease in the value of the positive secretory conditioned reflexes; in the second, it was less pronounced; thirdly, disinhibition was not by any means observed in every experiment, and, finally, as the tuberculous infection progressed, it did not increase as regularly as did the decrease in the positive secretory conditioned reflexes.

Treating the animals of the first group with streptomycin had a marked therapeutic effect; improvement of the animals' general conditon was observed which, judging from our observations on the dog Orsha, continued after treatement ceased. Restoration of the clinical indices was the first improvement effected by the continuous streptomycin treatment; normalization of the conditioned reflex food secretion did not occur until later. One can therefore assume that the disturbances which develop in conditioned food secretory reflexes during tuberculosis infection are reversible in the event of successful treatment.

The results of the treatment administered to the dogs of the second group were quite different. The course of the process induced by the more massive doses of the Koch's bacillus culture used in this instance was extremely severe, attended by acute intoxication and terminating in death, notwithstanding the active treatment of the animals. The issue of the disease also determined the dynamics of the pathology of nervous activity; during and after treatment, we observed in Pyatnistyi and Seraya progressive, acute, and, by this time, irreversible disturbances of the conditioned and unconditioned salivary reflexes.

SUMMARY

The author studied conditioned secretory reflexes in dogs in experimental tuberculosis prior to treatment and during streptomycin therapy. As established, tuberculosis provokes considerable disturbances of the brain function, manifested in depression of the positive conditioned salivary reflexes and in intensification of the process of sequential inhibition. The disturbance of differentiation was less regular in character. It was possible to reverse the functional changes occurring in the ceretonal cortex by streptomycin therapy. However, normalization of the indices of the conditioned reflex salivary secretion took place later than normalization of the clinical indices in the sick animals.

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LYSIS OF HUMAN AND ANIMAL FIBRINS BY THE PLAGUE MICROBE

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The incompleteness of our knowledge concerning the mechanisms allowing the unobstructed multiplication and spread of the plague agent in the host organism has forced the present authors to turn their attention to the study of fibrinolysins of the plague microbe, which to the present time have attracted the attention of only a few investigators. The first investigation of the fibrinolytic properties of the plague microbe was undertaken by Madison [16], who utilized the serum-free human and animal fibrin. In accordance with his data, the most sensitive to the action of the plague microbe fibrinolysin factors was rat fibrin, approximately six times less sensitive were human and guinea pig fibrins; even less sensitive were the fibrins of the ground squirrel and the cat, rabbit, cow, and finally, monkey. Horse, ram, and pig fibrins, according to Madison, were not lysed by the plague microbe.

Data concerning the presence of fibrinolytic properties in the plague microbe have been confirmed by a number of works [5, 6, 7, 11].

The research method for fibrinolytic properties, utilized by the authors of the mentioned studies, was disadvantageously different from Madison's method; the substrates for the action of the fibrinolytic factor were plasma clots rather than purified fibrin. The source of fibrinolytic activity, as in Madison's work, was a broth culture of the plague microbe.

By utilizing the mentioned method, A.S. Kvashina [5] has studied 20 cultures and found out that not all of the plague microbe cultures could dissolve human and animal plasma clots. Part of the cultures (6 out of 20) did not possess these properties. Without regard to the presence of fibrinolytic properties in the avirulent culture EV, the author arrived at the conclusion that the "avirulent cultures of Bact. pestis do not dissolve blood fibrin" and that the ability to produce fibrinolysin was resident in virulent cultures.

The data of A.S. Kvashina concerning the intensity of plasma fibrin dissolution from different animal types did not agree fully with Madison's results; Rat plasma fibrin appeared less sensitive to the fibrinolytic factor of the plague microbe in comparison to human and guinea pig fibrins. On the other hand, blood plasma clots from animals such as the horse and the ram, in a way similar to Madison's data, were not lysed even by the most active cultures. This permitted the author to compare the degree of animal susceptibility to plague infection with the susceptibility of blood fibrin to the fibrinolytic factor of the plague microbe. Here A.S. Kvashina referred to Hadfield's data [14] concerning the point that "the stability of blood fibrin is an indicator of unreceptivity of the animal to the corresponding infection".

Sera of animals immunized with cultures of Bact. pestis neutralize the action of the fibrinolytic factor from the plague microbe. This action of immune sera [6] is related to the presence of antifibrinolysins, "accumulation of which takes place gradually in a way similar to the formation of antibodies". However, these data do not solve the question concerning the nature of antifibrinolytic action of the immune sera, since immunization of the animals was conducted with whole microbial cells and not with a purified plague microbe fibrinolysin.

To the plague microbe fibrinolytic factor there is relegated a specific role in the pathogenesis of plague sickness. On the basis of literature data, and also on the basis of his own investigations, V.N. Lobanov [9] indicated that during plague sickness the inflammation foci contained no fibrin, or it was present in only small quantities. The author assumed that in the complex process of fibrinolysis, the fibrinolytic factor was not the only cause; however, its presence was without doubt.

The fibrinolytic factor dissolved the exudate fibrin from the inflammation focus, and thanks to this, the plague microbe spreads easily from the locus of penetration and from primary foci [4]. The opinion has been expressed that the value of fibrinolysins as pathogenic factors is exaggerated [3].

The nature and the mechanism of action of the plague microbe fibrinolytic factor have not been studied. Besides, the variability of results obtained by various authors dictated the necessity for a comparative investigation of the susceptibility of serum-free fibrin and plasma fibrin to the plague microbe fibrinolytic factor.

In the present investigation plasma and purified fibrins were utilized as substrates for demonstrating the fibrinolytic action of the plague microbe.

EXPERIMENTAL METHODS

In order to prepare blood plasma, the blood was mixed at bleeding with an equal volume of 1% solution of sodium citrate, or to 9 volumes of blood there was added 1 volume of 2.5% solution of potassium oxalate. The oxalated or citrated bloods were diluted with a physiological solution at the ratio of 1:5 and centrifuged. The plasma was stored at 5°.

Whole oxalated plasma generally served as the source of fibrinogen. The exception was human plasma, which was obtained from stabilized (stabilizer No. 7) donor blood.

Dry fibrinogen preparations were prepared by a single-stage precipitation with a saturated solution of sodium chloride and subsequent dialysis of the moist precipitate against a 0.5% solution of sodium citrate [8]. The fibrinogen that passed over into the solution was poured into ampoules and dried from the frozen state. The dry fibrinogen was also stored at 5°. Immediately prior to the experiment, the dry preparation was dissolved in a phosphate buffer (M 1/15, pH 7.4) and following fibrinogen determination, there was prepared a 0.1% solution.

The amount of fibrinogen was determined by Weinberg's method [2], as modified by the authors.

Cleaner fibrinogen preparations, relatively free from albumin admixtures, were prepared by Astrup's method* [12]. After a preliminary removal of plasma prothrombin (adsorption with trisubstituted calcium phosphate), fibrinogen was twice precipitated with ammonium sulfate. The precipitate was dissolved in veronal buffer (pH 7.8, ionic strength 0.05), and a preparation containing 5-10 mg of fibrinogen per ml was obtained. Such fibrinogen solutions were stable for two weeks of refrigerator storage.

Fibrinogen was coagulated by thrombin. The latter was obtained by activating the prothrombin with thromboplastin. A 5% suspension of fresh human brain in a phenolphysiological solution [17] served as the source of the thromboplastin factor. Purified prothrombin preparations were prepared from oxalated plasma by adsorbing it onto barium sulfate with subsequent washing of the adsorbent with 0.02 M acetate buffer and eluting it with 5% solution of sodium citrate. After dialysis against a physiological solution for 48-56 hours at 1-5° the eluent comprised a prothrombin preparation [10].

In the present investigation all experiments were carried out with a vaccination culture of Bact. pestis 17. In preliminary experiments it was ascertained that the fibrinolytic activity of this culture was sufficiently high.

The B. pestis 17 culture was grown at 28° for 48 or 20-24 hours on fluid and agar nutritional media. Agar media were prepared on Hottinger's broth. From among the fluid nutritional media there were utilized broths from digests of Hottinger and Martin, meat-peptone broth, and a phosphate broth according to Konikov [8], which was utilized by him for culturing of hemolytic streptococci with the idea of obtaining purified streptokinase preparations. There were conducted separate experiments with aerated broth cultures.

For the purpose of the fibrinolytic reactions, to various dilutions of the culture with the volume of 0.2 ml there were added 0.1 ml of 5% calcium chloride solution or thrombin, this depending on the substrate utilized (plasma or a 0.1% fibrinogen solution). After addition of 0.1 ml of the substrate, the contents of the test tubes were mixed rapidly and after clot formation they were placed in a thermostat at 37°. During the arrangement of the fibrinogenolytic reaction, the coagulant was added to the fibrinolysin-substrate system only after coagulation. Results of the fibrino-and fibrinogenolytic reactions were ascertained after 16 hours.

EXPERIMENTAL RESULTS

In the first experiments there were reproduced some data obtained by A.S. Kvashina [5]. The authors observed lysis of clots from plasmas of the human, the guinea pig, and the rabbit in the presence of centrifuged day-old broth cultures. Clots from plasma of the pig, ram, horse, and the bull were not lysed by even whole broth cultures. The highest fibrinolysin titres were given by poorly precipitable broth cultures; on the contrary, in those cases where the supernatant fluid was clear, lysis was limited by the first dilutions (1:2-1:4). The presence of bacteria has a direct influence on the duration of the reaction; this was completely contrary to A.S. Kvashina's opinion [5]. Further, the microbial cells were precipitated from the broth culture, and a suspension of them was prepared in a physiological solution. The results were completely clear: Microbe suspension produced lysis, and the microbe-free supernatant fluid did not possess fibrinolytic properties. It should be pointed out that in some cases plasma clots from human, guinea pig, and rabbit bloods could be partially and even completely lysed by some sterile broths, at the same time that plasma clots from other animals were always stable. This, evidently, could explain the contradictory data of A.S Kvashina concerning the filterability of the fibrinolytic factor. In the first communication A.S. Kvashina [5] noticed the ability of fibrinolysin to penetrate through biological filters; in her subsequent works she established that "after transfer through Chamberlaine [3] or Zeitz filters the fibrinolytic titre dropped sharply (from 1:32 - 1:64 to 1:1)" [6]. In the present experiments broth cul-

* The purity of fibrinogen preparations was checked by paper electrophoresis.

ture filtrates, as well as the supernatant fluid, did not produce lysis, whereas a bacterial cell suspension gave reproducible results. Fibrinolytic properties of microbes precipitated from broth cultures or grown on agar were the same. Three-fold washing of the bacteria with a physiological solution was not reflected in their fibrinolytic activity; this attested to the continuous connection of the fibrinolytic factor with the microbial cell. Fibrinolytic factor of the plague microbe was preserved after bacterial lyophilization.

The thermostability of the plague microbe fibrinolytic factor was high: Boiling in a water bath for 15-20 minutes did not lower its activity. In this respect, plague microbe fibrinolytic factor disclosed similarity to streptokinase, activity of which was lowered to one half by half hour heating at 65°; however, after further heating, even up to 100°, it was nearly unchanged [8]. Results of determinations of the thermoresistance of the plague fibrinolysins by A.S. Kvashina were completely contrary to present data: In her experiments heating at 60° for 10 minutes resulted in complete inactivation of the fibrinolytic factor [5].

Investigation of the susceptibility of the serum free fibrin and plasma fibrin to the plague microbe fibrinolytic factor exposed basic differences in the degree of lysis of their substrates.

Human and animal blood fibrins, obtained by coagulation of purified fibrinogen by homologous thrombin, was lysed by the plague microbe without regard to particular species. Plague microbe fibrinolytic factor lysed not only fibrin but also fibrinogen, which as a result of the lysis was transformed into a protein not coagulable by thrombin.

The susceptibility of unpurified human and animal plasma fibrin to the plague microbe fibrinolytic factor was different. Unpurified human, guinea pig, and rabbit plasma fibrin was lysed relatively easily by the plague microbe, whereas lysis of the unpurified pig and ram plasma fibrin could take place only in the presence of large numbers of bacterial cells, reaching several billions.

Stability against lysis during the action of the plague microbe was evidently related to the presence in animal blood serum of fibrin-protective factors, which were inhibitors of the blood fibrinolytic enzyme and are known in the literature as antifibrinolysins [1]. Concentration of fibrin-protective factors in blood of different animal types and the human differed. Thus, for example if in 1 ml of normal human plasma there were 79 antifibrinolysin units [13], then in 1 ml of bovine plasma there were 222 units [15]. In accordance with present data, the most active fibrin-protective factors were in pig and ram sera, less active in horse and bull sera. Addition of pig or ram serum to fibrin from any source made it more stable to fibrinolysin. Lability of human, rabbit, and guinea pig plasmas was possibly evidenced in a small content of fibrin-protective factors. Plasma antifibrinolytic properties, which were not observed by A.S. Kvashina and other authors, masked the true susceptibility of blood fibrin from animals unsusceptible to plague infection.

The present data let one state that there does not exist a connection between the susceptibility of various animal types to plague infection and the action of plague bacillus fibrinolysin on blood fibrin.

SUMMARY

Bact. pestis is able to lysate purified fibrin not only of human blood, but also that of animals investigated by the author (pig, sheep, horse, bull, dog, guinea pig, and rabbit). Antifibrinolysins, i.e., fibrin-protective factors, contained in the blood sera of various animals, depress fibrinolysis provoked by B. pestis. The fibrinolytic factor of B. pestis is connected with the microbial cell and does not pass into the nutritive medium.

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THE EFFECT OF DESOXYRIBONUCLEIC ACID ON THE RADIOSENSITIVITY OF α-CHYMOTRYPSIN IN AN ARTIFICIAL DNA–α-CHYMOTRYPSIN COMPLEX

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Because of the great biological importance of desoxyribonucleoproteins (DNP) and the comparative ease with which they are damaged by the action of ionizing radiation on the cell, in recent years the influence of ionizing radiation on these particular biological polymers has been investigated in vitro [3].

In these investigations injury to one of the components of the complex–desoxyribonucleic acid (DNA) –has been established. It has been shown by a comparative analysis of the radiosensitivity of DNA and DNP that the protein, entering into the composition of the DNP, increases the resistance of DNA to the action of radiation [8]. Until now, however, the possible influence of DNA on the radiosensitivity of the protein component of DNP has not been considered. The determination of this influence in the natural complex –the nucleoprotein– is impossible, for there is no method in existence which enables the specific biological activity of the histones and protamines, forming the main bulk of the protein of DNP, to be determined. This problem may, however, be solved by the use of artificial complexes of the enzyme protein with DNA. A comparison of the change in the activity of the enzymes in the free state and combined with DNA, under the influence of radiation, enables the influence of DNA on the radiosensitivity of the protein to be judged.

In the present research a complex of DNA with α-chymotrypsin was investigated. Damage to the protein after irradiation was estimated by the change in its proteolytic activity.

EXPERIMENTAL METHOD

DNA was obtained by the method of Mirsky and Pollister from the thymus and liver of cattle [10] and, by a slight modification of the method, from the pancreas [1], with subsequent deproteinization of the nucleoprotein by Sewag's method [12]; α-chymotrypsin was obtained by the method of Kunitz and Northrop [4]. The chymotrypsin was subjected to lyophilic drying or was preserved in 0.01N H_2SO_4 solution at a protein concentration of 4 mg/ml.

Complexes were obtained from the preparations of DNA and α-chymotrypsin; the reaction was carried out in a citrate buffer (pH 4.5) and in the same concentration of chymotrypsin as is present in the monomer state [2].

The DNA concentration was determined as phosphorus by the methods of Fiske-Subbarow or Spirin [5], and the nitrogen by Conway's method after mineralization with concentrated H_2SO_4 or by Nessler's micromethod. The molecular weight of the DNA was determined by the method of viscosimetry [6]. The proteolytic activity of the chymotrypsin and complexes was determined by Anson's method, by means of the amount of tyrosine liberated during incubation with substrate at pH = 7.6. After incubation for 20 minutes at 39° the protein was precipitated with 0.3N trichloroacetic acid solution and 2 ml of the filtrate was taken for determination of the tyrosine content with Folin's reagent in an alkaline medium. Irradiation was carried out with a RUM-7 x-ray apparatus at 60 kv, 20 ma, filter 0.5 mm Al and focus distance 75 mm.

EXPERIMENTAL RESULTS

Five preparations of complexes were obtained and their radiosensitivity studied. Their characteristics, and also those of the original DNA and α-chymotrypsin, are shown in Table 1.

We studied the action of various doses of radiation ranging from 50 to 50,000 r on α-chymotrypsin in the free state and combined with DNA. After the action of small doses no appreciable change was observed in the proteolytic activity of either the free enzyme or of the complex (Table 2). The variations in the results (±5%) were within the limits of error of the method.

It may be seen from the results obtained by the action of moderate doses (Table 3) that the α-chymotrypsin in a complex with DNA was more sensitive to the action of radiation than α-chymotrypsin in the same concentration in the free state. The proteolytic activity

TABLE 1. Characteristics of the Original Components and the Resulting Complexes

Preparation No.	DNA			α-chymotrypsin	N/P of DNA–α-chymotrypsin complex
	source from which obtained	molecular weight	N/P		
1	Pancreas	$1.8 \cdot 10^6$	1.9	Series No. 1, preparation kept in 0.01N H_2SO_4 solution	9.9
2	The same	$1.8 \cdot 10^6$	1.9	The same	9.2
3	The same	$3.5 \cdot 10^6$	1.84	Series No. 2, preparation kept in 0.01N H_2SO_4 solution	8.0
4	Liver	$1.6 \cdot 10^6$	1.89	Series No. 3, after lyophilic drying	6.9
5	Thymus	$4.0 \cdot 10^6$	1.76	Series No. 3, after lyophilic drying	7.7

TABLE 2. Action of Small Doses of Radiation on Proteolytic Activity of α-Chymotrypsin in the Free State and as a Complex with DNA

Dose (in r)	Preparation No. 1			Preparation No. 2		
	concentration of chymotrypsin during irradiation (in mg/ml)	proteolytic activity (as % of activity of unirradiated)		concentration of chymotrypsin during irradiation (in mg/ml)	proteolytic activity (as % of activity of unirradiated)	
		of free α-chymotrypsin	of α-chymotrypsin in complex		of free α-chymotrypsin	of α-chymotrypsin in complex
0	0.70	100	100	0.43	100	100
50	0.70	101	90	0.43	101	99
100	0.70	97	103	0.43	99	97
500	0.70	100	97	0.43	105	95

of the complex fell to 73% of the initial value when doses were used at which the activity of the free enzyme was not appreciably altered. With doses at which a fall in the activity of the free enzyme was observed, the activity of the complex fell to a much greater extent.

How may the greater degree of inactivation of chymotrypsin in the nucleoprotein complex be explained? It is presumable dependent on the disposition of the protein in the complex. Protein molecules, when they form complexes with DNA, are known to pass into a fully or partially opened state [7]. This shape of the protein probably facilitates inactivation during the action of radiation, by making the reactive centers more accessible to the action of free radicals formed by the radiolysis of water.

Inactivation may also be intensified as the result of preliminary partial denaturation, arising during the opening of the protein molecule in the process of complex formation. In this respect the findings in connection with alkaline phosphatase are of some interest, for its radiosensitivity increase sharply on its association with natural nucleoprotein [8], and the sensitivity of proteins to the action of radiation is also increased in a monomolecular layer, in which the protein is in the open state, as was observed in particular in experiments with pepsin [9]. It may therefore be considered that the cause of the greater radiosensitivity of protein associated with DNA is the open form in which the protein is present in the complex.

The fact that the radiosensitivity of an enzyme in the form of a complex is increased is interesting in connection with a further unsolved problem of radiobiology. Many enzymes are known to be much more resistant to irradiation in vitro than in vivo, although a considerable quantity of protective substances is present in the cell. The formation of a different group of complexes may become a method of increasing or weakening the radiosensitivity of individual biochemical components of the cell.

TABLE 3. Action of Moderate Doses of X-Rays on the Proteolytic Activity of α-Chymotrypsin in the Free State and as a Complex with DNA

Preparation No.	Concentration of α-chymotrypsin during irradiation (in mg/ml)	Dose (in r)	Proteolytic activity (as % of activity of unirradiated)	
			of free α-chymotrypsin	of α-chymotrypsin in complex with DNA
3	0.37	0	100	100
		10,000	98	91
		20,000	97	84
4	0.20	0	100	100
		10,000	98	91
		20,000	100	73
5	0.146	0	100	100
		25,000	65*	50
		50,000	42	26

* The radiosensitivity of chymotrypsin in this experiment was determined in a preparation stored for one year. It is not excluded that the radiosensitivity might be increased on account of partial denaturation during storage.

SUMMARY

The effect of DNA on the radiosensitivity of a protein included into the nucleoprotein complex was studied. Since biological activity, the change of which may conveniently serve as a criterion of protein disintegration, cannot yet be detected in the protein constituents of natural nucleoproteins, the authors used an artificial DNA–α-chymotrypsim complex for this investigation. It was found that after x-ray irradiation (10,000 and 50,000 r) of solutions of these complexes and of free α-chymotrypsim (control) the free enzyme is much more resistant to the action of the radiation than the same enzyme combined with DNA. The formation of different complexes may possibly be one of the factors which intensifies or reduces the radiosensitivity of the individual biological components of the cell.

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HISTOCHEMICAL DATA CONCERNING SOME METABOLIC DISTURBANCES IN ACUTE RADIATION SICKNESS

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The metabolism of radiation sickness has been studied basically by biochemical methods, which naturally could not give a complete picture concerning the microlocalization and character of changes of the studied processes in separate structural elements. Histochemical observations conducted by certain authors touched mainly on protein metabolism and first of all on nucleoprotein metabolism [5, 7, 10, 15].

For this reason the authors have conducted histochemical investigations of some enzymes, lipids, and glycogen in the lungs and the liver of rabbits subjected to general action of filtered x-rays in a dose of 800 r (LD-80/30). The lungs and the liver were chosen as the subjects for the study because of their large role in the pathogenesis of acute radiation sickness, because of their special participation in carbohydrate and lipid metabolism and also because of the lack of similar data in the literature.

EXPERIMENTAL METHODS

Twenty-five rabbits were utilized in the experiments; three rabbits served as controls and 22 rabbits were killed 6 hours, 1, 3, 5, 7, 10, 15, and 20 days after irradiation. Besides staining the sections with eosin-hematoxylin and scarlet, fat was differentiated in the sections by means of Sudan black and Nile blue, glycogen by the method of Shabadash, succinic dehydrogenase activity by the method of Shneider and Shelton, cytochrome oxidase activity by the method of Moog, and alkaline glycerophosphatase by the method of Gomori.

EXPERIMENTAL RESULTS

The researchers have shown that under conditions of acute radiation sickness there took place a basic disturbance in succinic dehydrogenase and cytochrome oxidase activities. Both of these enzymes play an important role in the final stages of biological oxidation of carbohydrates, fatty acids, and proteins. Succinic dehydrogenase serves as a carrier of hydrogen protons and electrons from succinic acid into the cytochrome system, thus catalyzing the transfer of succinic acid into fumaric. Cytochrome oxidase, by oxidizing cytochromes, serves as an intermediate carrier of hydrogen toward oxygen with the consequent formation of water.

The lung and liver succinic dehydrogenase and cytochrome oxidase activities changed monotypically, in complete conformity with the periods of radiation sickness development in the whole organism. After a slight drop during the first 2-3 hours after irradiation there occurred a short-term increase in the oxidative enzymes' activity. This was evidenced by an increase in cellular protoplasmic granules and by intensification of their staining. The cells were almost completely stained with dark blue color during development of cytochrome oxidase or dark violet during development of succinic dehydrogenase. This was especially noticeable in macrophages and in the bronchial epithelium.

After a day the enzyme activities decreased sharply, reaching a very low level at the climax of the sickness. In this period the enzymes were almost undevelopable and only in some cells could there be observed a small number of faintly stained granules. Beginning with the 10th day, cytochrome oxidase and succinic dehydrogenase activities gradually increased, and at the 20th day the activity almost reached the normal level.

In this fashion, evidently, important links in the intracellular oxidative processes were disturbed during radiation sickness; this without doubt played a large role in the lowering of tissue oxygen utilization and in a number of local and general disturbances. Thus, for example, it was established that in parallel with the lowering in the oxidative enzyme activity, there was a lowering in the digestive function of the macrophages, and this in turn led to disturbance in the lung and liver barrier functions [3]. Besides this, a considerable lowering of cytochrome oxidase and succinic dehydrogenase activities could be one of the reasons for accumulation of toxic metabolites.

In the literature there are contradictory meanings concerning the action of irradiation on tissue respiration. Data of a number of authors, as well as of the present ones, attest to basic changes in activity of oxidative enzymes during ray injury [8, 9, 11, 14, 19]; other, mainly biochemical, investigations, on the contrary, deal

with preservation basic enzyme systems of tissue respiration [1, 2, 16, 17, 18, 20, 22, 24, 25]. However, the divergence of present data from literature data was evidently related to the fact that in the biochemical studies in which there were investigated blood, biological fluids, tissue sections and homogenates, there were determined the total oxidative enzyme activity; histochemical methods let us study the state of various substances in separate structural elements. It is well known, and this was confirmed by the authors' previous works [4], that the distribution of enzyme activity in different structural elements of the same tissue is unlike and it changes differently under pathological conditions.

In this fashion, the present data do not contradict literature data, but on the contrary they supplement them, letting one judge more correctly the character of oxidative enzyme changes in the tissues of an irradiated organism.

In contrast to the oxidative enzymes, the activity of alkaline glycerophosphatase during the first days after irradiation changed slightly, but subsequently it increased considerably, remaining at the high level even during the recuperative period — on the 15-20th days (Fig. 1). Analogous observations were made by other investigators [21].

Glycogen investigations have shown that during the first hours after the action, the glycogen content decreased sharply; it was evidenced only in the central portions of the hepatic lobes (Fig. 2) and in individual cells of the bronchial epithelium and the cartilage, whereas liver glycogen was evidenced not only in hepatic cell protoplasm, but also in the lumens of intralobular capillaries and central veins. Thus it can be thought that in the beginning of sickness there takes place intensification of splitting and washing out of glycogen from the liver. Data of a number of biochemical investigations discuss this; these authors have observed development of hyperglycemia during the beginning of radiation sickness [2, 6, 13, 23].

At the end of the first day the liver glycogen was rapidly restored, remaining, however, lower than normal during the whole duration of the sickness. There was also a change in the character of glycogen distribution. During this period there was not noticed any significant difference in the glycogen content of the periphery and the center of the hepatic lobes; only in their different spots could there be observed separate groups of cells almost devoid of glycogen.

It is necessary to note that in some animals killed during the 7-10th day, the organ glycogen content was insignificant. Probably, such animals were killed during the preagonal period, when the glycogen stores decrease sharply [2].

The lowering of liver cell glycogen during the first hours after irradiation could be possibly tied in with the shock-like reaction arising as the result of irradiation, which is generally characterized by the sharp lowering of liver glycogen. Thus, such a lowering of glycogen is evidently not characteristic for all cases of radiation injury, but is connected with sharp reflector disturbances, produced by single massive external irradiation. In the case of acute injury by incorporated radioactive substances a similar decrease in glycogen in liver and in other organs during the first hours of the sickness does not take place [11].

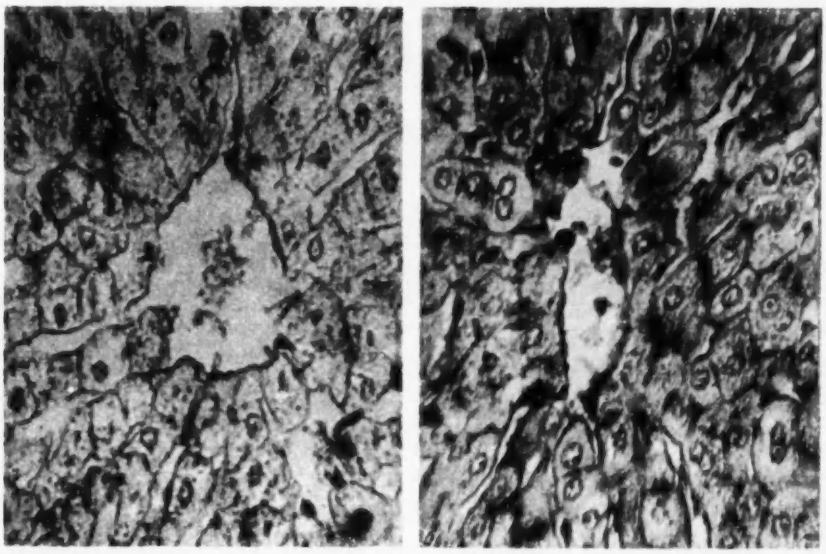

Fig. 1. Liver alkaline glycophosphate activity of normal (a) and on the 7th day after irradiation (b). Oc. 10×, ob. 40×. Staining according to Gomori.

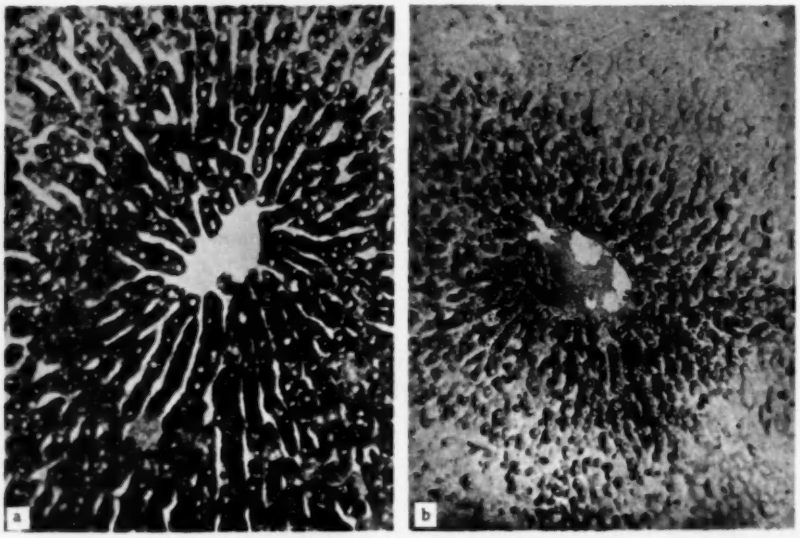

Fig. 2. Glycogen content in the liver of a normal rabbit (a) and at 6 hours after irradiation (b). Oc. 10×, ob. 8×. Staining according to Shabadash.

The disturbance of carbohydrate metabolism was not limited by the lowering of the stores of free liver glycogen. Absence of glycogen in considerable part of bronchial cartilage cells, and also increase in the obtaining intensity of fibrous structures and the interwoven substance attested to deeper changes in tissue carbohydrate metabolism of the irradiated organism. In the cells of the bronchial epithelium and in the macrophages there was a concurrent accumulation of large quantities of sugars, which were not removed from the tissues following treatment with preparations of salivary amylase. Such an increase in mucous cell and histocyte sugar could be related to the increased decomposition of tissue polysaccharides and disturbance of processes of their subsequent transformation in the cells.

Concurrently with glycogen changes there was a disturbance in lipid metabolism. Already observed at six hours after irradiation there was an increase in the content of liver neutral fat and an almost complete disappearance of phosphatides. In healthy animals phosphatides, appeared in liver cells in the form of separate globules, and after irradiation such globules disappeared; the protoplasm was stained diffusely in a gray-pink color. Analogous protoplasmic staining was observed during lipid development with Nile blue, scarlet, and also during the staining of the preparation with eosin-hematoxylin; it was most rapidl connected with changes of cellular lipo-and glycoproteins.

After a day the amount of neutral fat in hepatic cells increased greatly. This was indicated by a large number of pink droplets in the protoplasm of the majority of cells following staining with Nile blue. To an equal degree there was an increase in phosphatides, which also in a large number filled the protoplasm of hepatic cells. However, the increase in phosphatides was not prolonged and possibly was related to the tendency of the organism to restore the disturbed relationship between the processes of neutral fat hydrolysis and the synthesis of phospholipids. At the climax of the sickness the cellular neutral fat content remained high and the number of phosphatides fell below normal. Such a decrease in phosphatides could be partially dependent on the increased activity of alkaline glycerophosphatase during this period of the sickness.

If the animals survived the acute phase of radiation sickness (10 days), the phosphatide content of liver cells increased gradually; the neutral fat appeared as before in large quantity. During this the character of fat distribution changed. If during the first days and at the climax of the disease the fat appeared mainly in peripherally situated cells, then during the 15-20th days it was as a rule observed in the central portion of the lobes. Such a distribution of fat permitted speculation on various mechanisms of lipid metabolism disturbances in these or other periods of radiation sickness. Elevated fat content of lobular periphery in the beginning of the sickness indicated simple adiposity, which could be connected with the lowering of liver glycogen and disturbance in the processes of digestion of the surplus neutral fat entering the liver. Appearance in such a case of protoplasmic fat decomposition evidently did not play an important role.

Observation of lipids primarily in the central portion of the hepatic lobes was a sign of fatty dystrophy of the hepatic cells. Such a change in hepatic cells in latter

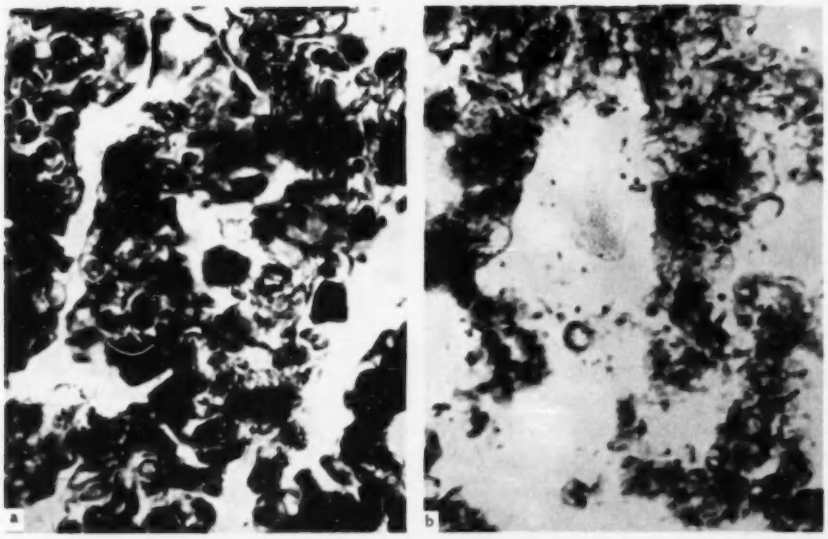

Fig. 3. Lung phospholipid content of a normal rabbit (a) and on the 7th day after irradiation (b) Oc. 10 ×, ob. 40 ×. Staining with Sudan black.

periods after irradiation developed most rapidly as the result of general metabolic disturbances during radiation sickness, while one of the primary reasons for this could be the appearance of exhaustion as the outcome of the sickness.

The disturbance of lipid metabolism in the lungs took place in a different manner. During the whole period of the sickness neutral fat did not appear in the cells of lung tissue, with the exception of cartilage cells; the content of phosphatides decreased sharply (Fig. 3). At the same time there was an appearance of fat–which stained blue with Nile blue–in septal cells, macrophages, and bronchial epithelium. Their appearance could be connected with the lowering of oxidative enzyme activity at the climax of the sickness; as the result there was an accumulation of incompleted oxidized products of fat cleavage, which were formed in the liver. With the increase in oxidative enzyme activity, the content of incompletely oxidized products of fat cleavage was decreased and there were observed essentially phosphatides.

In this way, the presented materials attest to the essential disturbances of some metabolic phases of lung tissue and liver cells. Evidently, the most highly expressed disturbances occurred in lipid metabolism, while the glycogen content during the course of the sickness remained at a level sufficient to maintain the activity of the irradiated organism. Only during the first hours after irradiation was there a sharp decrease in the liver glycogen content, which possibly could be one of the causes of the early lethal results of radiation sickness. This is supported by the acute destruction of liver in animals perishing in the first few hours after irradation.

With regard to the changes in metabolism of separate lipid fractions, the observations show that under the influence of irradiation there was a many-fold increase in liver neutral fat content and in the incompletely oxidized by-products of fat cleavage in the lungs. At the same time the quantity of phospholipids was decreased significantly.

SUMMARY

Carbohydrate and lipid metabolism, as well as the activity of alkaline glycerophosphatase, cytochrome oxidase, succinic dehydrogenase, and succinic dehydrogenase, were studied histochemically in the liver and lungs of irradiated rabbits. The activity of succinic dehydrogenase and cytochrome oxidase drops considerably in animals with acute radiation sickness, whereas the activity of alkaline glycerophosphatase is markedly elevated. In addition, a considerable increase of the neutral fat content in the liver, with accumulation of underoxidized products of its splitting in the lungs. The amount of phospholipids is usually markedly reduced in both organs. The amount of glycogen in the liver changes but little; only during the first hours after irradiation and in the dead animals is a loss of the glycogen store in the liver noted.

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DEPENDENCE OF PHAGOCYTIC ACTIVITY OF LEUCOCYTES ON RESPIRATORY PHOSPHORYLATION

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Glycogen contained in leucocytes plays a basic role in assuring their physiological function. Proofs of the dependence of leucocyte mobility on the presence of glycogen in them were given by K. Voskresenskii [2] and Avice [7]. In relation to the leucocyte phagocytic activity it has been established that one of the ways in which this function is guranteed with energy is glycolysis; this was evident from the experiments of N.V. Puchkova [4] and O.S. Sherstneva [6] who have observed a lowering of phagocytic activity following elimination of leucocyte glycolysis with monoiodoacetic acid, and also from the experiments of Fleischmann [8], who obtained the same result by eliminating leucocytic glycolysis by means of sodium flouride. Cleavage of glycogen within the leucocytes, according to data of I.F. Seitz and coworkers [5], takes place also via amylolysis with a subsequent respiratory phosphorylation of the glucose formed. Investigations by Fleischmann have shown that elimination of leucocytic respiration by means of cyanides lowered their phagocytic activity. These facts let one suppose that energy for phagocytic function is assured not only by glycolysis, but also by respiratory phosphorylation; however, direct proofs of respiratory phosphorylation's participation in this process are absent.

EXPERIMENTAL METHODS AND RESULTS

There was investigated, in invitro experiments, the influence of eliminating the respiratory phosphorylation by means of 2,4-dinitrophenol (DNP), dissolved in Ringer's solution for warm-blooded animals, on the human leucoctye phagocytic activity. Phagocytic activity was determined by the method of G.E. Platonova [3]. There were conducted eight experiments, each accompanied by a control. The experiments have shown that DNP in the concentration of $1:10^{-3}$ sharply decreased the leucocyte phagocytic activity - on the average from 39 to 8% (Fig. 1a).

In the authors' previous experiments on the exclusion of glycolysis by monoiodoacetic acid, the leucocyte phagocytic activity was lowered from 44 to 25%, i.e., to a considerably lesser extent than following exclusion of respiratory phosphorylation. This one to suppose that under aerobic conditions respiratory phosphorylation plays a more essential role in assuring energy for leucocyte phagocytic activity than does glycolysis.

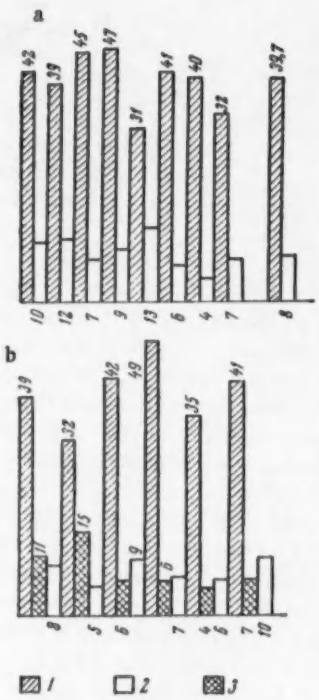

Fig. 1. Influence of 2,4-dinitrophenol on phagocytic activity of leucocytes (a) and the influence of ATP on phagocytic activity of leucocytes previously subjected to exclusion of respiratory phosphorylation (b). 1) Control 2) DNP ($1:10^{-3}$) 3) ATP($1:10^{-3}$).

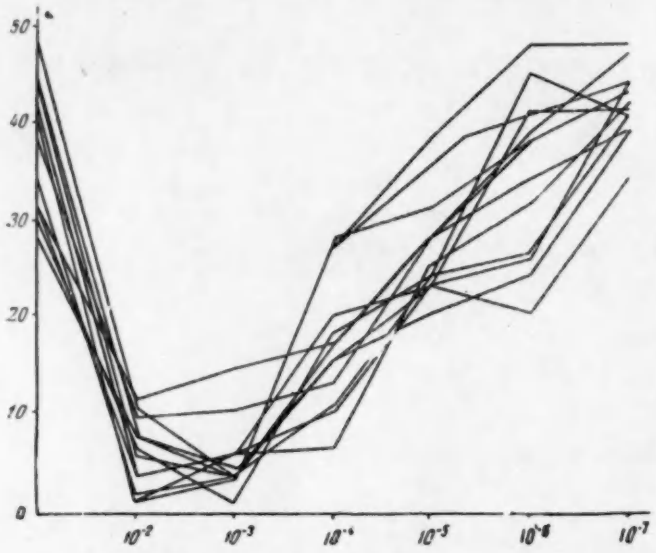

Fig. 2. The influence of ATP on the phagocytic activity of leucocytes (abscissa concentration of the sodium salt of ATP, ordinate-percent of phagocytizing leucocytes).

The attempt to restore leucocyte phagocytic activity which was previously lowered by exclusion of respiratory phosphorylation, by adding a sodium salt of ATP, was unsuccessful; even a large concentration of ATP ($1:10^{-3}$) produced only an insignificant increase in phagocytic activity of leucocytes poisoned with 2,4-DNP. this increase was within the accuracy limits of the method (Fig. 1b).

The influence of various ATP concentrations on phagocytic activity of leucocytes not previously subjected to the exclusion of respiratory phosphorylation was investigated in 12 experiments. These experiments have shown that during intact leucocytic metabolism concentrations of ATP from $1:10^{-2}$ to $1:10^{-4}$ lowered phagocytosis (Fig. 2). From this follows that the phagocytic process is slowed down by the surplus of phosphate macroergs, which was created by the addition of ATP to the medium. The stimulatory action of ATP in the quantities which were noticed in the work of D.N. Alpern and R.U. Lipshitz [1], were not observed in the present study; this is possibly due to the fact that these authors experimented with exudate leucocytes, whereas the present experiments were conducted on blood leucocytes.

SUMMARY

As shown experimentally (in vitro), exclusion of respiratory phosphorylation in the leucocytes considerably reduces their phagocytic activity. Addition of ATP to the medium results in restoration of this function. The addition of ATP to the medium in a concentration not under $1:10^{-4}$ tends to depress phagocytosis, if leucocyte metabolism remains intact.

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FURTHER OBSERVATIONS ON THIAMIN DEHYDROGENASE

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The existence of thiamin dehydrogenase, previously described by A. A. Titaev [1-3], has recently been put in doubt by the work of S. É. Shnol' [5,6], who investigated extracts of various organs and tissues and also the blood plasma and came to the conclusion that an antithiamin dehydrogenase activity was present and that the thiamin dehydrogenase reaction was not enzymatic in nature. The main argument against A. A. Titaev's findings is the observation by S. É. Shnol' that increased thiochrome formation is present in the plain control tube and the control tube preliminarily inactivated by boiling, by comparison with the experimental tube. In his reply to S. É. Shnol's findings as described in the latter's first paper [5], A. A. Titaev [4] pointed out the incontestability of the presence of thiamin dehydrogenase in the blood of patients with hypertension. S. É. Shnol's second investigation [6], using S^{35}-thiamin and keeping to the technique used by A. A. Titaev, failed to reveal the enzymatic nature of the thiamin dehydrogenase reaction, although in this paper the author did not reply to A. A. Titaev's claim that thiamin dehydrogenase activity could clearly be detected in the serum of patients with hypertension. The question of the enzymatic nature of this process under consideration thus remains unresolved.

In order to shed light on this problem, we investigated the thiamin dehydrogenase activity of the cerebrospinal fluid and the serum in various diseases.

EXPERIMENTAL METHOD AND RESULTS

The thiamine dehydrogenase activity of the cerebrospinal fluid was investigated in 40 children suffering from tuberculous meningitis. Assuming the enzymatic nature of this reaction, and in view of the relatively low protein content of the cerebrospinal fluid, in each experiment we took 1 ml of fluid, and we adhered to all the molar proportions of the ingredients of the incubation mixture recommended by A. A. Titaev.

In all cases, after incubation the thiochrome content was higher in the control than in the experimental sample. Boiling one of the experimental tubes slightly increased the formation of thiochrome therein, but the quantity of this compound was equal to that found in the control tube without CSF in only two of 20 cases.

The results obtained relating to the "antithiamin dehydrogenase" action of the CSF are given in Table 1.

It may be seen that the "antithiamin dehydrogenase" activity of the CSF varied between relatively wide limits.

During the investigation of the thiamin dehydrogenase activity of the serum obtained from healthy subjects and patients with different diseases, the experiment was always performed in three tubes with different volumes of serum (0.05-0.1-0.2 ml) and two control tubes were used, one without serum and the other containing 0.1 ml of serum, inactivated on a water bath. In the majority of samples tested (in 14 of 15 healthy subjects; in five patients with hepatitis; in eight with gastritis; in 8 of 9 with peptic ulcer; in 15 with malignant neoplasms, and in 18 of 20 with other diseases), the enzymatic nature of the thiamin dehydrogenase reaction could not be demonstrated, for the quantity of thiochrome found in the controls was greater than or equal to that in the experimental tubes.

Two groups of patients, however, gave reproducible and quite definite results suggesting activation of the thiamin dehydrogenase reaction by the blood serum. This refers to patients with hypertension and, more especially,

TABLE 1. Thiochrome Content after Incubation of Tubes for 20 Hours (as % of that Found in Controls)

Tube no.	TC*	Tube no.	TC*	Tube no.	TC*
1	72	15	69	29	70
2	80	16	92	30	48
3	58	17	81	31	69
4	60	18	84	32	92
5	66	19	74	33	84
6	90	20	87	34	71
7	74	21	71	35	80
8	75	22	64	36	80
9	80	23	80	37	59
10	56	24	92	38	62
11	52	25	91	39	90
12	74	26	57	40	85
13	78	27	99		
14	39	28	65		

*TC = Thiochrome content.

TABLE 2. Quantity of Thiochrome Formed in a Period of 20 Hours (in γ)

Patient	Diagnosis	Test I (0.05 ml)	Test II (0.1 ml)	Test III (0.2 ml)	Control I (without serum)	Control II (boiled serum)
B-r	Uremia	24	25	36	24	25
M-a	"	39	38	48	30	36
K-ch	Tumor of the kidney	66	72	64	54	59
I-j	Uremia	66	78	84	70	80
S-k	"	45	49	58	40	49
A-a	"	72	79	89	66	70
K-a	Hypertension (stage III)	42	43	74	51	50
M-v	" "	38	45	62	34	32
Ts-v	" "	55	72	60	57	49
A-o	" "	60	67	83	52	54
P-v	" "	41	39	59	38	32
S-i	" "	29	37	44	35	33
Ya-ya	Hypertension (stage II)	62	66	52	50	52
L-m	Hypertension (stage I)	34	36	33	45	38
B-o	" "	47	58	46	45	44

to patients with uremia developing as the result of chronic nephritis. Another noteworthy fact was that patients with far-advanced forms of hypertension gave an increased thiamin dehydrogenase activity. In these experiments the thiochrome formation increased obviously with an increase in the volume of serum used in the test, and preliminary boiling of the serum partially or completely suppressed activation of thiamin dehydrogenation. It must be mentioned that isolated sera, taken from patients with stage I or II hypertension, often gave the opposite—i. e., "antithiamin dehydrogenase"—effect. These findings are given in Table 2.

In a proportion of the tests depression of the thiamin dehydrogenase reaction was observed, beginning with the tubes containing 0.2 ml of serum. It is probable that the relationship between the activators and the inhibitors of the thiamin dehydrogenase reaction is highly complex, so that their interaction varies even after the simple further dilution of the experimental sample with phosphate buffer. Thus, when five experiments were carried out with equal volumes of all the components, but with different final volumes of incubation fluid (3 and 5 ml), a difference was observed in the results. Two tests out of five, which did not show thiamin dehydrogenase activity in the more concentrated medium (3 ml), did show such activity when diluted to 5 ml.

The results obtained suggest a possible connection between the thiamin dehydrogenase activity and an enzymatic mechanism provided that the thermal denaturation of proteins at pH=7.4 can be considered to be a sufficiently reliable criterion. The relationship between the activators and inhibitors of thiamin dehydrogenation in the serum is not yet clear and requires special investigation. Attention is directed to the slight increase in thiochrome formation in the samples of serum taken in uremia and inactivated by boiling. This points to the presence of certain nonprotein activators of the thiamin dehydrogenase reaction in the serum.

SUMMARY

Examination of the cerebrospinal fluid and blood serum in persons with different diseases in most cases failed to reveal any thiamin dehydrogenase activity. It was only in uremia and, for a time, in hypertensive vascular disease that the blood serum possessed a pronounced ability to activate the dehydrogenation of thiamin in the presence of adrenalin.

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CONCERNING S. É. SHNOL'S ARTICLE "ON THE EXISTENCE OF THIAMIN DEHYDROGENASE"

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In his investigation [8], S. É. Shnol' has once again repeated the initial stage of my research with unpurified extracts and serum, the activity of which may vary within wide limits, as a result of which a sufficiently wide range of concentrations must be used for its detection. S. É. Shnol' used only one concentration, which was three times higher than that used by me [2, 3], and he carried out the reaction in the presence of adrenalin. It must not be forgotten that in these conditions the final effect of the reaction is determined by its first stage – the oxidation of adrenalin – and a negative result is dependent not on the "antithiamin dehydrogenase" invented ad hoc by S. É. Shnol', but on the delay in the oxidation of adrenalin by the tissue inhibitors [5]. It must also be remembered that a phosphate buffer at pH = 7.2-7.4 is not a satisfactory medium for thiamin dehydrogenase [2,3]; in this pH zone, in the presence of adrenalin, a Ringer-bicarbonate solution should be used. Furthermore, the method of chromatographic separation in an acid mixture of protein-containing incubates, without preliminary treatment, used by S. É. Shnol', may be a source of error as a result of the adsorption of thiochrome on the protein. Doubts also arise concerning the technique of preparation of the extracts of the pituitary and thyroid glands, whose weight is only a few milligrams. No factual data is given in the article.

Thiamin dehydrogenase is widely distributed in the animal and plant worlds; its existence has been proved in experiments with purified enzyme solutions and by the use of adrenochrome [4, 6]. Thiamindehydrogenase is readily found in organ extracts after removal of inhibitors and ballast substances [6]. Indirect evidence of its existence may be seen in the result of the work of A. Ya. Rozanov [1] and of Iacono and Johnson [9].

From his results, obtained in conditions unsuitable for the detection of thiamin dehydrogenase, S. É. Shnol' makes an unjustifiable conclusion. Only one conclusion is possible from these results: In the conditions selected by S. É. Shnol', thiamin dehydrogenase is not found in extracts or in serum from rats. Obviously, on the basis of the foregoing remarks, it is difficult to detect this enzyme in such conditions. In conclusion, it is necessary to refute S. É. Shnol's claim that in his work he used my own instructions. If he had followed these instructions, these mistakes would not have arisen in his work.

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ONCOLOGY

ON THE ANTIGENIC STRUCTURE OF ROUS SARCOMA

Communication II. Viral and Tissue Antigens of Rous Sarcoma

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Using the reactions of complement fixation and anaphylaxis with desensitization, the presence of two specific antigens – viral and tissue – was shown in certain tumors of viral origin (for example, Shope's cancer and papilloma) [1, 4]. In our experiments [2] using the reaction of precipitation in agar, only one specific antigen was discovered in Rous chicken sarcoma. The extract of this sarcoma and its antiserum, preliminarily depleted normal chicken serum, and a mixture of extracts from organs of a healthy chicken (liver, kidney, spleen, muscle), and the sarcoma MX-659, induced by a carcinogen, formed only one line of precipitation.

It remained unclear as to which of the antigens, viral or tissue, corresponds to that line. This question was studied in the present investigation.

EXPERIMENTAL METHOD

In the experiments we used extracts, in a physiological NaCl solution, of Rous sarcoma, transplanted MX-659 sarcoma, initially induced with methylcholanthrene, from the organs of a healthy chicken (liver, kidney, spleen, lungs, muscle), normal chicken serum, and also rabbit serum immunized to Rous sarcoma. The preparation of the extracts from tumors and normal tissue and the immune serum was described by us earlier [2].

Fractionation of the Extract of Rous Sarcoma. The extract of the sarcoma was centrifuged at 30,000 rpm (60,000 g) for 1½ to 2 hours. As a result of centrifugation the fluid divided into 4 layers: the upper – a small layer of fat and lipoproteids, the middle– supernatant fluid, the lower – sediment; on the dense lower sediment was situated a layer, barely visible to the eye, of loose sediment, which, with the slightest agitation, easily dispersed. The supernatant fluid was carefully drawn off, by means of a pipette, from the middle layer, and studied in the experiments. The dense and loose sediments were carefully mixed in the remaining supernatant fluid by a rapidly turning pestle, fastened to the axle of an electromotor. Then the suspended sediment was restored to its original volume with a physiological solution of NaCl (pH 7-7.2) and again centrifuged, at the same speed as the first time, for a period of 30 minutes. The supernatant fluid was discarded, and the sediment, resuspended in the original volume of the physiological solution of NaCl, was again subjected to ultracentrifugation according to the same regime as in the second time. The centrifugation was carried out in the domestic centrifuge UTsP – 1 at 4-6°. The supernatant fluid was drawn off with a pipette; the sediment was rinsed with physiological saline and resuspended in the saline in the proportion of 1/2 to 1/8 of the original volume of the extract of Rous sarcoma.

Thus, we obtained two fractions from the extract of Rous sarcoma: 1) supernatant fluid, taken from the middle layer, and 2) dually precipitated sediment. The sarcoma extract and the fractions obtained from it were tested for carcinogenic activity by intracutaneous inoculation of chickens, 5-6 months of age, of the white leghorn family (Table 1).

TABLE 1. The Carcinogenic Activity of the Extract of Rous Sarcoma and Its Fractions

Fraction of the extract of Rous sarcoma	Final dilution of the Rous sarcoma virus						
	per se	1:10	1:100	1:250	1:500	1:1000	1:5000
Extract	+	+	+	+	+	+	–
Precipitated sediment	+	+	+	+	+	–	–
Supernatant fluid	–	–	–	–	–	–	–

Symbols: plus) Appearance of tumor; minus) absence of tumor.

The Acquisition of Purified Specific Precipitating Antibodies to Rous Sarcoma from the Original Sera. For the acquisition of purified specific precipitating antibodies we took advantage of the method devised by Z. A. Avenirovaya and G. I. Abelev for the isolation of antibodies from a precipitate formed by an organ-specific antigen from the liver of mice and its corresponding antibody, and also an antibody to the specific antigen of mouse hepatoma.

The method of obtaining purified specific precipitating antibodies consisted of the following. First the original serum to Rous sarcoma was completely depleted of its antibodies to normal chicken serum, to antigens from the organs of healthy rats, and to the sarcoma MX-659. The method of depletion of the serum of Rous sarcoma was described by us in detail in a previous work [2]. The serum, depleted to Rous sarcoma, was centrifuged at 60,000 g for 30 minutes to afford complete liberation of the normal and tumor tissues, taken for the depletion, from the intracellular components (microsomes, mitochondriae, their membranes, etc.). Along with the intracellular components, the remnants of the precipitate, formed in association with the depletion of the serum, were withdrawn in the sediment.

For the acquisition of the specific precipitate to the antisarcoma serum, depleted in the same manner, we added the following fraction from the extract of Rous sarcoma – supernatant fluid, following ultracentrifugation, practically devoid of corpuscular, biologically active virus. To 1 ml of depleted antisarcoma serum 0.2-0.3 ml of this fraction was required. The mixture, consisting of depleted antisarcoma serum and the virus-free fraction of Rous sarcoma, was incubated for $1\frac{1}{2}$ hours at 37° and 16-18 hours at 4°. The resulting specific precipitate was divided by centrifugation at 4,000 g for 10-15 minutes. The supernatant fluid, i.e., antisarcoma serum, was neutralized with both normal and tumor (sarcoma MX-659 and Rous sarcoma) tissues, studied for the reaction of precipitation in agar, and also investigated for viral-neutralizing activity (Table 2, 1B). The specific precipitate was washed three times with large volumes of a physiological solution of NaCl (pH 6.6-6.8) using centrifugation at 4,000 g for 5-10 minutes.

Following the washing of the precipitate with physiological saline the latter was discarded, and a phosphate-citrate buffer (pH 3.2) was added to the precipitate in the proportion 1/8 in relation to the original volume of antisarcoma serum. In addition, a considerable portion (up to 15-20%) of the specific precipitating antibodies was separated, which yielded one zone of precipitation with the antigens of Rous sarcoma and did not react with the antigens from the organs of healthy chicken and from sarcoma MX-659. This precipitate was eliminated by centrifugation, and the supernatant fluid, in which the separated specific precipitating antibodies were present, was carefully neutralized with dry sodium carbonate (Na_2CO_3) to a pH of 7-7.2. Subsequently, for brevity in the presentation, we will call the eluted specific precipitating antibodies the eluates against Rous sarcoma. The latter were tested for virus-neutralizing activity and by the reaction of precipitation in agar. In all we obtained 9 different samples of the eluviate against Rous sarcoma from two rabbit antisarcoma sera (No. 865 and 912, and mixtures of them). The eluates against Rous sarcoma obtained from the original serum No. 865 were the most active when tested by the reaction of precipitation in agar.

EXPERIMENTAL RESULTS

The results of the 2 separate experiments, carried out for the purpose of testing the fractions isolated from the extract of Rous sarcoma, are presented in Table 1. As can be seen, the sediment from the Rous sarcoma obtained after its two-fold rinsing in physiological saline by means of centrifugation at 60,000 g gave rise to tumors in chicken in a titer of 1 : 500. The supernatant fluid formed following ultracentrifugation did not bring about the appearance of tumors in the chickens. Thus, we obtained a fraction with the corpuscular, biologically active virus of Rous sarcoma, and a fraction which did not contain it.

As has already been shown [2], the sera against Rous sarcoma, depleted with normal chicken serum and a mixture of the extracts from the organs of healthy

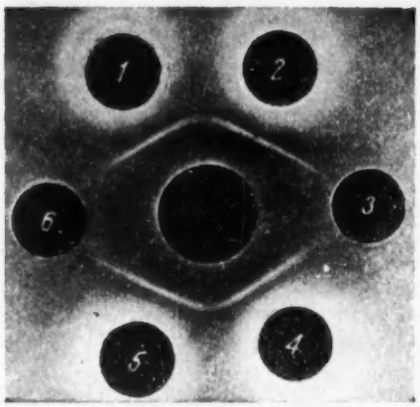

Fig. 1. Results of the reaction of precipitation in agar. Depleted antisarcoma serum No. 865 (in the center). Depletion was carried out with normal chicken serum and a mixture of the extracts from the normal organs of a chicken (liver, kidney, spleen, muscle) and sarcoma MX-659. 1,4) Antigens from the extract of Rous sarcoma; 2,5) antigens from the supernatant fluid, not containing corpuscular, biologically active virus; 3,6) antigens from the doubly precipitated sediment which was centrifuged at 60,000 g.

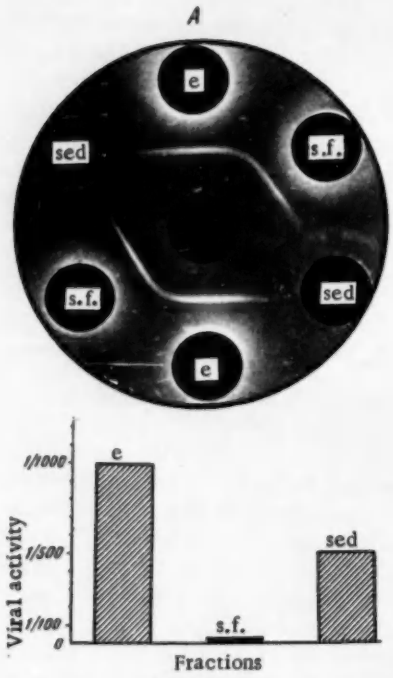

Fig. 2. Distribution of the precipitating antigen and the Rous sarcoma virus. A) Reaction of precipitation in agar (in the center—purified, specific precipitating antibodies to Rous sarcoma); e) Rous sarcoma extract; s.f.) supernatant fluid of Rous sarcoma (60,000 g); sed) doubly precipitated sediment of Rous Sarcoma (60,000 g).

chickens and from sarcoma MX-659, reacted only with the extract of Rous sarcoma. Testing these sera with fractions obtained from the extract of Rous sarcoma showed that a specific precipitation zone was noted with both the Rous sarcoma extract and the supernatant fluid following high-speed centrifigation (60,000 g for $1\frac{1}{2}$ to 2 hours). In contrast to this, the doubly precipitated sediment did not react with the depleted antisarcoma serum in the same manner (Fig. 1). That picture was presented by eluates to Rous sarcoma (Fig. 2.). Following the removal of the specific precipitating antibodies from the depleted antisarcoma serum, the latter no longer reacted with the Rous sarcoma extract and its fractions.

On the basis of the data presented it would be possible to infer that the specific zone of precipitation is caused either by the dissolved viral antigen or by the tissue-specific protein arising from the reaction between the virus and the tissue of the chicken.

Simultaneously with the reaction of precipitation in agar, the fractionated sera against Rous sarcoma were tested by the neutralization reaction. The results from one of the 5 experiments are presented in Table 2. The serum against Rous sarcoma (No. 865), depleted with normal chicken serum and with a mixture of extracts from the organs of a healthy chicken and sarcoma MX-659 (see Table 2, IA), and that same serum (see Table 2, IB) from which the precipitating antibodies against Rous sarcoma were also removed, neutralized the virus in the same manner as the original antisarcoma serum (see Table 2, I), lowering the titer of the virus by 50 times. In the other experiments the titer of the virus was lowered from 40-100 times. The eluted precipitating antibodies against Rous sarcoma (see Table 2, IC) did not possess virus-neutralizing activity, which affords us a basis for thinking not of the dissolved viral antigen, but of the tissue-specific protein.

Thus, the serum against Rous sarcoma contains 2 types of specific antibodies: first, virus-neutralizing, and second, precipitating a specific tissue antigen of Rous sarcoma.

A specific tissue antigen of Rous sarcoma was discovered, in our experiments, which yielded a zone of precipitation with the original antisarcoma sera, the eluates against Rous sarcoma, and the sera against Rous sarcoma, preliminarily depleted with normal chicken serum and with mixtures of the extracts from the organs of a healthy chicken and sarcoma MX-659. On the basis of the data presented it is difficult to judge the nature of the specific antigen demonstrated by us, inasmuch as it could be either a specific tumor protein which arises

TABLE 2. Neutralization of the Virus of Rous Sarcoma by Antiserum Depleted by Various Methods

Fraction from serum No. 865 against Rous sarcoma	Dilution of the Rous sarcoma virus						
	1:10	1:50	1:100	1:250	1:500	1:1 000	1:5 000
I	+	—	—	—	—	—	—
I A	+	—	—	—	—	—	—
I B	+	—	—	—	—	—	—
I C	+	+	+	+	+	—	—
Physiological saline	+	+	+	+	+	—	—

Symbols: plus) Appearance of tumor; minus) absence of tumor.

in the presence of viral action on the tissue of the chicken, or an antigen of mucin-like substance which is copiously produced in association with the growth of Rous sarcoma. Experiments performed with the antigen from mucin-like material showed that an antigen is contained within it which corresponds to the specific antigen of Rous' sarcoma.

In conclusion, we note that we were unsuccessful in obtaining a specific zone of precipitation for the virus antigen in the reaction of precipitation in agar, apparently due to an insufficient concentration of the virus or an insufficient amount of the virus-neutralizing antibodies. The possibility has not been excluded that further concentration of the Rous sarcoma virus and the antibodies against it might lead to the acquisition of a specific zone of precipitation for the virus antigen. Experiments elucidating the nature of the specific antigen of Rous sarcoma are continuing.

SUMMARY

The following constituents were obtained in fractionating Rous' sarcoma: 1) supernatant fluid (60,000 g; 1.5-2 hours), which did not contain corpuscular biologically active virus; 2) doubly reprecipitated precipitate, provoking tumors in chickens in the 1 : 500 titre. Purified specific precipitating antibodies were isolated from the precipitate formed by the specific Rous sarcoma antigen (supernatant fluid). Testing of these antibodies in reaction of precipitation in agar has demonstrated that they react with the supernatant fluid in the same way as with the Rous sarcoma extract and do not react with the doubly reprecipitated precipitate; they did not neutralize sarcoma virus. Consequently, specific precipitating antigen of Rous sarcoma is not a virus antigen and is evidently of tissue origin. Apart from the specific precipitating antibodies, the virus neutralizing antibodies were revealed in the Rous sarcoma antiserum. These antibodies neutralized the virus at the same titre, as the initial Rous sarcoma antisera. However, they did not react in precipitation reactions in agar with any of the fractions of the Rous sarcoma tissue.

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A COMPARATIVE INVESTIGATION OF THE ACTION OF PREPARATIONS FROM THE GROUP OF CHLORETHYLAMINES AND ETHYLENEIMINES ON EXPERIMENTAL HOMO- AND HETEROTRANSPLANTED TUMORS

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Contemporary oncology deals with only a small number of the experimental forms equivalent to the neoplasias of the human. This essentially complicates the search for new active antitumoral preparations. Therefore, the interest and the attempts of a number of investigators to use the method of heterotransplantation of human tumors for experimental chemotherapy is understandable.

Thanks to the use of influences suppressing the immune powers of the recipient organism (cortisone, roentgen irradiation), it has been possible, in the last few years, to transplant human tumors – sarcomas and carcinomas – to hamsters and rats for prolonged periods, and to pass them serially over periods for several months and years [2,10,14,15].

In the years 1956-1958 there appeared the first short reports on the action of various chemopreparations on animals with tumores of human derivation [9,11,12,13].

It was established in the experiments of these authors that the different chemotherapeutic agents act variously on the heterotransplants of the same human tumor.

In the construction of therapeutic experiments on animals with heterotransplanted tumors it is possible to anticipate three variations in the results:

1. Tumors existing under unfavorable conditions within the alien organism (deteriorating blood supply, immunological effects) become vulnerable to any influences. In this case tumor heterotransplants are a poor means for the selection of new active antitumoral preparations or for the study of their spectra of activity.

2. Tumors developing resistance to the preparations to which they were sensitive in the original host. Resistance of this type could be explained by a change in the biological properties of the heterogenic tumor. In this case, investigating various preparations, we would not be able to elucidate their real activity.

3. The sensitivity of the tumors to the chemopreparations being preserved or changing minimally subsequent to heterotransplantation. A result of this type would speak in favor of the approach involving laborious investigations on heterotransplantations of tumors from the point of view of using them for experimental and clinical chemotherapy.

The purpose of this work was a comparative study of the action of certain preparations on several animal tumors under conditions of homo- and heterotransplantation. In the experiments we used a number of compounds from the group of chlorethylamines and ethylenimines, whose antitumoral activity, as well as methods of administration and dosages in association with homotransplantation, were thoroughly studied by coworkers in the Laboratory of Experimental Chemotherapy of the Institute of Experimental and Clinical Oncology, AMN SSSR [3,5,6,7].

EXPERIMENTAL METHODS

Many authors have studied the heterotransplantation of experimental tumors. They have obtained contradictory results. The results of the majority of these investigators are presented in the reviews of the literature by Yu. M. Vasil'ev [1] and Ahlstrom [8]. We considered it necessary to ourselves to study the following tumors, the potential and rapidity of their growth under the conditions of the alien organism, their weight and dimension: 1) rat tumors, transplanted to mice receiving cortisone: sarcoma M-1, sarcoma 45 (typical form and form resistant to sarcolysin), sarcoma SSK, Gehren's carcinoma, Walker's carcinosarcoma, ascitic hepatoma (strain AGF); 2) mouse tumors, transplanted to rats receiving cotisone: Ehrlich's ascitic tumor, lymphosarcoma – strain LIO-1, two strains of mammary gland cancer – RSM and RMZh, Crocker's sarcoma, squamous cell cancer of the esophagus – strains OZh-1 and OZh-5, sarcoma 298, hepatoma 22; 3) Brown-Pearce's tumor of rabbits, transplanted to rats and mice receiving cortisone.

A concise characterization of the strains of experimental tumors used by us is contained in the review by E. E. Pogosyants [4].

* Yu. M. Vasil'ev, our elder scientific coworker, took part in the direction of the work.

All of the mouse tumors were transplanted, by means of cell suspensions, to nonpedigreed rats, 3-4 weeks of age: the rats tumors were transplanted to nonpedigreed mice, 15-20 grams in weight; the Brown-Pearce rabbit tumor was transplanted to mice of the S_3Na line, 15-20 grams in weight, and to nonpedigreed rats, 3-4 weeks of age (30-40 grams in weight). The site of transplantation for all the tumors was the subcutaneous cell layer of the left flank.

The following tumors, subsequent to heterotransplantation, usually did not exceed the measurements of a pea and became necrotic early: sarcoma M-1, sarcoma 45 (typical form and form resistant to sarcolysin), sarcoma SSK, Gehren's carcinoma, Walker's carcinosarcoma, ascitic hepatoma AGF, lymphosarcoma LIO-1, Ehrlich's ascitic tumor, squamous cell cancer of the esophagus OZh-1 and OZh-5, hepatoma 22.

Adequate growth, subsequent to heterotransplantation to rats, was yielded by the following mouse tumors: mammary gland carcinoma (strains RSM and RMZh), Crocker's sarcoma and sarcoma 298, and also Brown-Pearce's rabbit tumor (with transplantation to mice and rats). We used these strains in our therapeutic experiments. The effect of the following preparations was studied: embichin, i.e. methyl-di-(2-chlorethyl)-amine, in a single dose of 0.15 mg/kg for rats and mice; sarcolysin, i.e. DL-p-di-(2-chlorethyl)-amino-phenylalanine, in doses of 0.5 mg/kg, 1.5 mg/kg, and 2 mg/kg; metasarcolysin fluoride, i.e. 2-fluoro-5-di-(2-chlorethyl)-aminophenylalanine, in a single dose of 1.6 mg/kg for rats, 5 mg/kg for mice, and 4 mg/kg for rabbits; benzodate, i.e. 2-benzyloxy-4,6-diethyleneimino-1-3-5-triazine, in doses of 8 mg/kg for rats, 15 mg/kg for mice; thio-TEPA (triethylenethiophosphoramide), in doses of 2.5 mg/kg for rats and 4 mg/kg for mice; phenamete, i.e., di-(2-chlorethyl)-aminophenacetyl-methionine ethyl ether, in doses of 20 mg/kg for rats and 110 mg/kg for mice. Because of the varying tolerance of the rats, mice and rabbits to the preparations employed by us, we used them in the maximum tolerable doses.

Tumor therapy using these preparations began on the 5th day following heterotransplantation and continued over the course of 7-8 days, after which the animals were sacrificed, using ether. All the preparations were administered intraperitoneally, with the exception of phenamete, which was introduced into the stomach. The animals received benzodate at intervals of 72 hours; the remaining preparations were administered daily. The average diameter of the tumors in the control and experimental groups were measured on the 5th, 8th and 12th day after transplantation. At the end of the experiment the percent inhibition of tumor growth was calculated by the average weight of the tumors of each group. All the animals were injected with cortisone twice a week – 2.5 mg for the rats, and 2 mg for the mice. For comparison and as control, therapeutic experiments were set up simultaneously on homotransplants of these same tumors. Treatment with the preparations in these experiments continued for 10-12 days.

The data obtained are presented in the table. As can be seen, sarcoma 298, subsequent to homotransplantation, was sensitive to phenamete and sarcolysin (89 and 82 percent inhibition). The high degree of sensitivity of this tumor was preserved in association with heterotransplantation as well (95-89% inhibition). Sarcoma 298, under the conditions of homotransplantation, was absolutely insensitive to embichin. The same observation was made with heterotransplantation of the tumor to rats.

Crocker's sarcoma in mice was sensitive to benzodate in a dose of 15 mg/kg (95% inhibition); under conditions of heterotransplantation to rats that preparation gave rise to 68% inhibition of tumor growth in a dose of 8 mg/kg. A certain reduction in the antitumoral effect possibly depends on the smaller dose and the shortened interval of treatment. Using sarcolysin in doses of 2 and 1.5 mg/kg, the growth of Crocker's sarcoma in mice was inhibited by 59 and 50%. With heterotransplantation of the tumor to rats, sarcolysin in these doses gave rise to 66 and 64% inhibitions respectively. Sarcolysin, in a dose of 0.5 mg/kg, did not effect the growth of Crocker's sarcoma, either in mice or in rats.

Strain RMZh (cancer of the mammary gland) was essentially not inhibited by the application of sarcolysin and phenamete either in homo- or heterologous transplantation.

Sarcolysin fluoride showed itself to be a preparation strongly influencing the growth of another mammary gland carcinoma (RSM) under conditions of homo- and heterotransplantation (97 and 81%). Embichin and thio-TEPA did not act on this tumor in mice; in rats, thio-TEPA caused a stimulation in the growth of Crocker's sarcoma, while embichin did not evidence a notable effect.

Preservation of the sensitivity of the Brown-Pearce tumor to sarcolysin and metasarcolysin fluoride was observed in the case of transplantation of that strain to animals belonging to two different genuses. Thus, metasarcolysin fluoride yielded 94% inhibition of the growth of that tumor with subcutaneous transplantation of it to rabbits, 81% inhibition with the tumors growing in mice, and 82% in rats. Sarcolysin (in doses of 3 mg/kg after 72 hours in the experiments on rabbits and 1.5 mg/kg daily in mice and rats) did not give rise to a statistically significant inhibition in the growth of this tumor, neither with homologous nor heterologous transplantation.

In conjunction with histological investigations of the heterotransplanted tumors, a morphological picture was observed identical with that of the original. In tumors treated with the active preparations there were noted degenerative changes, up to necrosis of the tumor cells, increase in the amount of connective tissue stroma, and lymphoid and leukocytic infiltration. The detailed description of the microscopic picture of the treated heterogenic tumors will be the subject of a special report.

Thus, the investigation carried out showed that the degree and character of sensitivity of tumors of mice (mammary gland carcinomas, strains RSM and RMZh, Crocker's sarcoma, sarcoma 298) and Brown-Pearce's

Results of the Therapeutic Experiments

Tumor	Preparation	Homotransplantation: number of animals	dose of the preparation (in mg/kg)	average weight of the tumor (in mg)	percent inhibition	criteria R*	Heterotransplantation: number of animals	dose of the preparation (in mg/kg)	average weight of the tumor (in mg)	percent inhibition (-) or stimulation (+)	criteria R*
Mouse sarcoma 298	Control	10	—	1 566	—	—	10	—	1 995	—	—
	Phenamete	10	110	166	89	1	10	20	115	—90	1
	Control	10	—	1 566	—	—	14	—	1 943	—	—
	Sarcolysin	10	1,5	270	82	1	15	1,5	202	—89	1
	Phenamete	10	110,0	156	89	1	17	20,0	94	—95	1
	Control	10	—	1 566	—	—	15	—	935	—	—
	Sarcolysin	10	1,5	270	82	1,0	15	1,5	66	—93	1,0
	Embichin	9	0,15	1 477	5	0,44	14	0,15	1 050	+12	0,621
Crocker's mouse sarcoma	Control	5	—	5 190	—	—	14	—	7 339	—	—
	Benzodate	6	15	343	95	0,998	18	8,0	2 332	—68	1
	Sarcolysin	—	—	—	—	—	13	1,5	3 336	—54	1
	Control	10	—	3 850	—	—	15	—	2 056	—	—
	Sarcolysin	9	2,0	1 544	59	0,999	13	2	688	—66	0,998
	"	9	1,5	1 888	50	0,991	—	—	—	—	—
	"	9	0,5	3 311	19	0,6	14	0,5	2 623	+27	0,715
Mouse carcinomas RMZh	Control	10	—	955	—	—	10	—	1 400	—	—
	Sarcolysin	9	1,5	755	19	0,669	9	1,5	1 372	—2	0,236
	Phenamete	8	110,0	537	43	0,872	10	20,0	1 032	—26	0,669
Mouse carcinomas RSM	Control	10	—	2 060†	—	—	19	—	2 714	—	—
	Thio-TEPA	10	4	1 680	18	0,619	18	2,5	3 838	—41	0,979
	SF**	—	—	—	—	—	18	1,6	491	—81	1
	Control	10	—	2 255	—	—	19	—	3 271	—	—
	SF**	10	5,0	52	97	1,0	—	—	—	—	—
	Embichin	9	0,15	2 211	2	0,3	16	0,15	2 798	—14	0,6
Brown-Pearce tumor in rats	Control	5‡	—	2 850	—	—	10	—	3 210	—	—
	SF**	5	4	140	94	0,998	10	1,8	570	—82	0,999
	Sarcolysin	5	3 over 72 hr	2 440	5	0,4	10	1,5	2 280	—29	0,669
Brown-Pearce tumor in mice	Control	—	—	—	—	—	10	—	411	—	—
	BPT††	—	—	—	—	—	9	5	75	—81	1,0
	Sarcolysin	—	—	—	—	—	9	1,5	350	—14	0,617

* Criteria R of less than 0,975 indicates a non-significant difference from the control. † The experiment was set up jointly with S. A. Papoyan.
‡ The tumor was transplanted under the skin. ** SF=Sarcolysin fluoride. †† BPT=Brown-Pearce tumor.

rabbit tumor to preparations of the chlorethylamine and ethylenimine groups (embichin, sarcolysin, metasarcolysin fluoride, phenamete, benzodate, thio-TEPA) is preserved with heterotransplantation to rats.

SUMMARY

The author studied the effect of some antiblastic chemotherapeutic preparations on the growth of mouse tumors (Crocker's sarcoma, cancers RSM and RMZh of the mammary gland, and sarcoma 298) and rabbit Brown-Pearce tumor in heterotransplantation. Mouse tumors were inoculated into three- and four-week-old rats, and rabbit tumors were inoculated into rats and mice. Cortisone was administered to the recipient animals. Therapeutic experiments were conducted on homotransplants of the same tumors. It was shown that the homo- and the heterotransplanted tumors were approximately identical in sensitivity to chemotherapeutic preparations investigated (embichin, sarcolysin, thio-TEPA, etc.).

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CHARACTERISTICS OF THE TRANSPLANTABLE CARCINOMA OF THE RAT MAMMARY GLAND (RMK-1)

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(Presented by Active member of AMN SSSR A.D. Timofeevskii)
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The transplantable mammary gland carcinoma is obtained primarily in mice and rats and is widely utilized in experimental oncology for ascertaining some common mechanisms of tumor growths and for working out methods of therapy.

There are indications that the rat mammary gland carcinoma, in comparison with mouse mammary gland carcinoma, possesses more pronounced reactivity to some therapeutic methods; partially, in its reactivity to methods of hormonotherapy, it is similar to the reactivity of human mammary gland carcinoma [6, 7]. This similarity allows us to presuppose that the utilization of rat mammary gland carcinoma as an experimental model could be more perspective in investigations concerning methods of hormonotherapy than utilization of mouse mammary gland carcinoma. Besides, rats are more resistant to infections and various actions, which likewise served as a preference for utilizing them in experimental investigations.

Meanwhile, it is known that in rats, as against mice, there exists a small number of transplantable mammary gland carcinomas. Thus, in agreement with the summary of Dunham and Stewart [2], in rats there are only seven mammary gland carcinoma cultures, and in the USSR, according to the summary of E.E. Pogosyantz, there is only one such culture. The authors were successful in finding in the literature only five works [3, 4, 5, 8, 9] in which there were descriptions of the transplantable rat mammary gland carcinoma.

EXPERIMENTAL METHODS AND RESULTS

In 1958, in the authors' laboratory, there was obtained a transplantable rat mammary gland carcinoma, it was called RMK-1 (rat mammary gland carcinoma). The original tumor was observed in a thoracic mammary gland of a pregnant female 10 months of age (up to that moment the female raised five litters). It is of interest that during the last pregnancy the tumor grew very rapidly. However, after parturition, at the time of lactation, the tumor became ulcerous and almost completely resorbed; in the place of the tumor there remained only a small scarred surface with thickened borders. Soon after termination of the lactation the tumor relapsed and after two months its diameter reached approximately 4 cm; after this the tumor was transplanted subcutaneously, as a suspension, into two-week old females and males.

Macroscopically the tumor was solid with small hemmorhagic foci and insignificant necrosis. Microscopically the tumor appeared as a characteristic for rat alveolar carcinoma, composed of wide traces of poorly differentiated epithelial cells, divided by a small amount of stroma. The tumor cells were small and dark with slightly eosinophilic protoplasm, the nucleus was cyst-like, poor in chromatin. Mitoses were rare. In the epithelial rods there was observed a large number of pseudoglandular nodules with formation of the so called cribrose structures. In the tumor there were observed separate large cystoid nodules, however, nipple-like and nodular formations were absent. The secretory activity of the tumor was expressed moderately. The tumor's stroma was porous, with a large number of cellular elements; in the periphery there were observed lymphohistocytic infiltrations (Fig. 1 and 2).

During the first generation the tumor took in two out of three females. The tumors grew slowly and after 6 weeks reached only about 5 cm in size. During the second generation the tumor was also transplanted into female and male rats, two and three weeks of age. The tumor took in 32 out of 42 females (76.1%) and in 17 out of 19 males (89.4%). In the third generation the tumor was transplansplanted into females and males, three-and four weeks of age; it took in 17 out of 23 females (73.9%) and in 23 out of 31 males (77.4%).

The rate of tumor growth during the second and third generations did not differ from the first. It should be noted that the tumor grew somewhat more rapidly in males than in females. During two weeks after transplantation, the tumor in females and males reached on the average 0.2-0.5 cm after 4 weeks 0.8;,-1.5 cm; after 6 weeks, 1-2.5 cm, and after 8 weeks, 2-4 cm. Spontaneous resorption of tumors reaching the dimensions

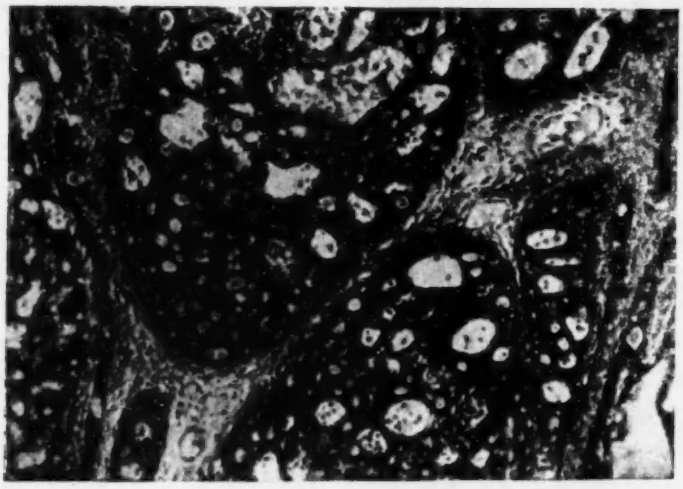

Fig. 1. Poorly differentiated alveolar carcinoma of the rat mammary gland. Large compartments of tumor cells divided by porous stroma. Inside compartments - pseudo-glandular cysts (cribrose carcinoma) containing a small amount of secretions and sloughed cells. Staining with eosin-hematoxylin. Magnification 130 x.

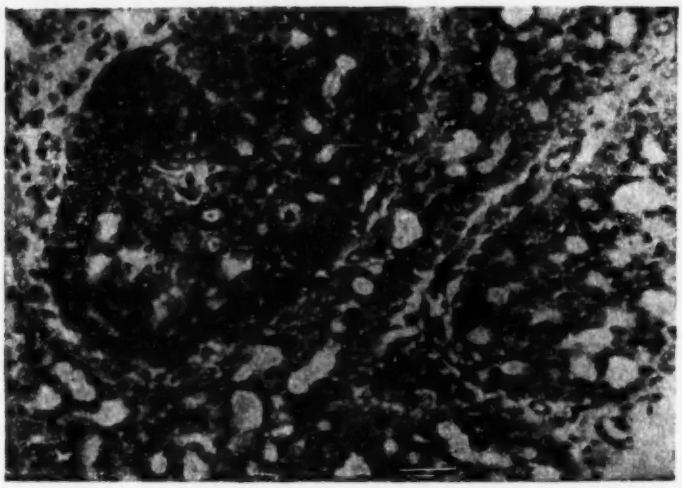

Fig. 2. The same tumor as in Figure 1. The tumor cells are small, dark, with a small amount of protoplasm and with a light cyst-like nucleus. Staining with eosin-hematoxylin. Magnification 270 x.

of 0.5-1 cm was not observed. The life duration of rats with tumors was 2.5-4 months.

There has been obtained the eighth generation of the tumor RMK-1, transplantable to females and males -weanling rats. Essential changes in the percent of transplantability, and also in the macro-and microscopic structure of the tumor during seven generations were not observed; however, the rate of tumor growth became somewhat more rapid. In the seventh generation the mean tumor sizes in 2,4 and 6 weeks after transplantation were correspondingly 1,2 and 3 cm.

In a number of conducted experiments it was established that RMK-1 possesses a clearly expressed reactivity to hormone actions, and it should be expected that the obtained tumor will become of definite interest for experimental oncology.

SUMMARY

Spontaneous carcinoma of the rat mammary gland of unknown origin, which appeared in a normal adult female rat, was inoculated subcutaneously to other rats and, thus, a transplantable RMK-1 carcinoma of

the mammary gland was obtained. At present this carcinoma passed through the 8th generation. The "take" of the tumor 79.2%. The tumor is transplanted to female and male weaning rats, grows slowly and does not metastasize. Rats with the tumor survive up to 4 months. Microscopically, the tumor represents an alveolar low-differentiated carcinoma characteristic of rats. Although the principal strain remained alveolar up to the 14th generation, in some subpassages the tumor was of cystopapillary and solid structure.

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THE HISTOGENETIC ACTIVITY OF SUBSTANCES SECRETED BY THE TRANSITIONAL EPITHELIUM

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(Presented by Active Member of the AMN SSSR G. V. Vygodchikov)

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The incontrovertible facts dealing with the high osteogenetic activity of transitional epithelium [1-8] naturally pose the question of whether or not there is a secretion from this tissue of an active histogenetic factor, free of the living cells. Discussion may be directed toward the examination of the histogenetic activity in the nonliving mucosa of the organs of urinary excretion, toward the secretion of active extracts from the transitional epithelium, and, finally, toward the testing of the products of secretion which the transitional epithelium develops under experimental conditions [4-6].

In the current work material is presented from the experimental investigations in these three directions.

EXPERIMENTAL METHOD

The urinary bladder mucosa of guinea pigs was cut into small pieces with scissors, and pellets were made from the obtained paste, approximately 3 mm in diameter. The latter were fixed in 96° alcohol or acetone for 12-24 hours. Then the pellets were dried in a vacuum dessicator (those fixed in alcohol were preliminarily washed in acetone), moistened with serum, and implanted in the anterior wall of the stomachs of the guinea pigs. After 8-22 days the implants were fixed. In place of fixation, some of the pellets prepared according to the indicated method were placed in test tube and subjected to five-fold freezing in acetone and thawing. Then they were implanted in the anterior peritoneal wall and fixed afer 5-18 days. For the preparation of the extracts the mucosa of the urinary bladder in the guinea pigs was again cut into small pieces with scissors and pulverized in an ebonite mortar. The homogenate, in which the cells were not observable microscopically, was suspended in an equal volume of physiological saline and,each day for 7 days, injected with a syringe into the same site on the anterior wall of the peritoneum. On the 10th day that portion of the peritoneal wall was fixed. Extraction of the mucigenous portion of the urinary bladder in the guinea pigs was carried out in an aqueous medium—either physiological saline or distilled water containing 1% guinea pig blood serum. The mucosa ground with a pestle was mixed with double its volume of physiological saline or distilled water. The homogenate was centrifuged at 1200 revolutions per minute for 10 minutes. The clear supernatant fluid was injected daily for 10 days into the same site in the anterior peritoneal wall. The control guinea pigs were injected with homogenate or extract of liver, prepared in the same manner. The extracts were prepared in the cold; penicillin and streptomycin were added to the material injected into the animals. The site of injection was fixed after 4-12 days.

For the investigation of the products of secretion of transitional epithelium in adult rabbits,the vessels of the left kidney were ligated. Under such conditions urine is not formed, and the transitional epithelium of the pelvis produces a secretion, directed, in particular, into the cavity of the pelvis [5].

After 10 days the left ureter was ligated at the middle third and cut above the site of ligation. The ends of the ureter were separately sutured to adipose tissue. The contents of the pelvis were drained off via the site of drainage of the central end; the drainage site of the peripheral end served as a control. Both subjects were fixed after 10—30 days. In the project a total of 57 animal-recipients were used. The types of histological fixatives utilized were acetone, alcohol-formol, and Gel' *solution. The sections were stained with hematoxylin-eosin, by the method of Dominichi-Kedrovskii using the PAS-reaction, and by the method of Gomora to alkaline phosphatase.

EXPERIMENTAL RESULTS

Implantation of the pellets prepared from the mucosa of the urinary bladder did not lead to epithelial growth in a single case. A proliferative reaction of the connective tissue was observed in the area surrounding the implant, along with its gradual resorption, the character and rapidity of which depended on the method by which the pellet was prepared. Implants fixed in

alcohol or acetone underwent resorption slowly (3-5 weeks) with the participation of coarse, miltinucleated simplasts. It was characteristic that even after 12-17 days a slight degree of alkaline phosphatase activity was preserved in the fixed implants, while in the surrounding connective tissue the alkaline phosphatase reaction was invariably absent. In association with the implantation of fixed urinary bladder mucosa in the tissues,the usual reaction to a foreign body was observed in all cases. Resorption of the mucosa, implanted after freezing and thawing, occurred more rapidly—within the first 10 days. The process was accompanied by a minimal reaction, with a small number of macrophages, predominantly mononuclear. At the site of the implant having undergone resorption, there remained fibroblastic tissue with a small amount of collagenous fibers. Osteogenesis was not observed in a single instance.

The absence of osteogenetic activity on the part of the fixed mucosal tissue of the urinary bladder does not contradict the data [7] on the obtaining of chondrogenesis in rabbits at the site of implantation (in the ear or the subrenal capsule) of fixed urinary bladder wall. The positive results in these experiments were dependent, as the authors correctly assumed, upon the induction activity of fixed muscle tissue of any origin, and not upon the osteogenetic properties of transitional epithelium.

With repeated daily administration of the homogenates, as well as aqueous and saline extracts, of the urinary bladder mucosa, into the anterior peritoneal wall of guinea pigs, similar results were observed in all cases. Manifest thickenings formed at the site of injection on the 4-5th day. They became less diffuse, but, in places, denser, by the 8-10th day. Histologically, these thickenings consisted of reactive connective tissue. The main mass of their cellular elements was made up of coarse branching cells with basophilic cytoplasm and a large nucleus containing a prominent nucleolus. Mitoses were frequently observed among these cells. A large number of spherical polyblasts were present here, in the cytoplasm of which there appeared a polysaccharide which did not ferment amylase.

A thin polychromatic (stained by the method of Dominichi-Kedrovskii) granularity appeared in the basophilic cells with the contracted branchings on the 5-8th day, and, simultaneously, there appeared glycogen and a polysaccharide which did not ferment amylase.

Neighboring on these elements were disposed small foci of young myeloid cells and more mature cells of the granular leukocytic series- basophilic promyelocytes, eosinophilic promyelocytes, myelocytes, metamyelocytes, and, finally, eosinophilic and basophilic leukocytes. It was possible to clearly show the stage of conversion of the spherical, polyblastic type cells into the coarse, polychromatophilic, myeloid elements.

The generating foci of extramedullary hematopoiesis were usually distributed in the intermuscular connective tissue and, also, in the site of fatty tissue, With the accumulation of the polychromatic granules in the polyblasts, i.e., with their conversion into myeloid elements, glycogen appeared in them in the form of a compact mass, dispersed in the peripheral portions of the cytoplasm. The young myeloid elements, with the not-yet-segmented nucleus, clearly differed from the agglomerated round-nucleated cells of the lymphocyte and from the plasma cells in their alkaline phosphatase activity. In such foci of extramedullary hematopoiesis there were not encountered disintegrating leukocytes with degenerating nuclei, and when stained for polysaccharides it was not possible to find extracellular masses of glycogen, although the mature leukocytes present here were very rich in this polysaccharide.

All this distinguished these foci from the accumulation of leukoctyes which can arise subsequent to the insufficient observation of aseptic technique.

Besides the foci of hematopoiesis, actively proliferating connective tissue was also formed in the sites of injection of the extracts, in which the processes of fiber formation were initiated. The cellular elements presented here were mainly coarse fibroblasts with basophilic cytoplasm. Collagenous fibers formed around the cells, creating homogeneous discs, here and there, of the osseoid type. However, the appearance of osteoblasts or the development of typical osseoid structures was not observed in a single case. We also never observed any epithelial cells in the sites of injection which would be able to indicate their contamination in association with the introduction of the material. Extracts of the liver did not give rise to marked thickenings in the site of injection. In this case only a proliferative reaction of the connective tissue was observed, without the formation of foci of hematopoiesis.

The experiments involving the drawing off of the secretion produced by the transitional epithelium in the cavity of the pelvis with ligation of the renal vessels was accompanied, in a portion of the animals, by the suctioning off with a syringe of the contents of the ureter that exited from those kidneys. The rather viscous clear fluid obtained, when tested for polysacharides (in smears), yielded an intensive PAS reaction, which was not eliminated by amylase. In addition to this, the fluid contained granules of glycogen in large number. Thus, the fluid corresponded to the contents of the pelves in the kidneys with the ligated vessels which was observed in the histological preparations, and also to the contents of the cysts in the transplants of transitional epithelium [4-6]. We named the secretion obtained pelvin [5].

At the site of drainage of the lower end of the ureter (control) no formation of bone or myeloid tissue occurred. In this location there developed only the usual granulation tissue, with a small number of fibers and small foci of round-nucleated lymphoid elements. It should be kept in mind that in rabbits the transplantation of urinary bladder mucosa to connective tissue does not cause the induction of osteogenesis [6].

In contrast to this, at the site of drainage of the upper end of the ureter, via which pelvin drained from the pelvis, the formation of myeloid tissue had already occurred by the 20th day. The development of multinucleated simplasts was noted here, with large quantities of lipoids and a finely granulated polysaccharide, stable to amylase, around which there arose foci of myeloid hematopoiesis similar to those which were formed at the site of injection of the extracts. Even in this case osteogenesis was not observed in a single instance. The fact that the generation of myeloid tissue precedes the formation of the giant cell simplasts is evidence for believing that it actually develops from local elements instead of the fatty tissue to which the transected ureter was sutured.

Thus, non-living transitional epithelium does not possess any kind of specific histogenetic activity.

It should be considered that in association with the death (fixation) of the tissue, substances, contained in the transitional epithelium and responsible for the induction, are destroyed. They, apparently, are also destroyed in conjuction with the slow resorption of the epithelium rendered dead by freezing and implanted in the connective tissue.

At the same time, the homogenates and extracts of the transitional epithelium, prepared in physiological saline and in distilled water, demonstrated, upon repeated injection, the ability to induce myeloid hematopiesis from the local connective tissue cells. This same effect also gave rise to the flow of a secretion from the transitional epithelium (pelvin) into the connective tissue. Thus, in normal transitional epithelium, as well as in the products of its secretion, substances are contained ensuring the myelogenetic activity of that tissue even without contact with living epithelial cells.

However, the injection of extracts and pelvin is insufficient to cause the appearance of osteogenic tissue. They induce only myeloid hematopoiesis, genetically closely related to osteogenesis. This is either due to the fact that all the factors necessary for osteogenesis, are not contained in the extracts of transitional epithelium and pelvin or to the fact that, in addition to chemical factors, osteogenesis also requires the mechanical action of growing epithelial cysts and extensions in the surrounding connective tissue.

SUMMARY

Physiological saline and water extracts of urinary bladder mucosa and secreta of transitional epithelium (pelvin) possess histogenetic activity. They induce myeloid hemopoiesis at the site of injection. There is no histogenetic activity in fixated and frozen tissue of vesicular mucosa.

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THE ROLE OF HALOIDS (BROMINE AND IODINE) IN THE METAMORPHOSIS OF AMPHIBIA

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The ideas of several authors [5,14] on the special role of the hypophysis in the metabolism of bromine and the formation of the bromine hormone in the hypophysis has, at the present time as well as earlier [8], given rise to considerable doubts. Numerous investigations [2,3,4,11,12,13,16,17,19,20,22], using the method of tagged atoms, have conclusively shown that the only site of accumulation of bromine in the organism is the thyroid gland, and that only there can bromine be detected in the composition of the protein. These data obtained in relation to bromine have long been known in regard to iodine. However, if the role of iodine in the function of the thyroid gland is more or less known, then this cannot be said of bromine, despite the fact that in several investigations [7,8,9] a relationship has been pointed out between the histogenesis of the thyroid gland and the introduction of external bromine.

As is well known, one of the most sensitive indicators of the activity of thyroid gland hormones is the metamorphosis of amphibia. The ideal subject in this case is the axolotl (the neotonic form of Amblystoma mexicanum), since the axolotl, under natural conditions, does not possess the capacity of independent metamorphosis. Thus, any indications of the conversion of the axolotl to its adult form—amblystoma – must be regarded as a result of the experimental activity [6].

The purpose of the present work was to compare whatever influence bromine and iodine showed on the metamorphosis of the axolotls.

EXPERIMENTAL METHOD

We used 52 axolotls in the experiment, 8-10 months of age (of these, 40 axolotls were of the black race, and 12 white), drawn from the eggs of one laying.

Two series of experiments were carried out on 20 axolotls, which had been transferred to 4 aquariums, 5 in each. The third series included 12 axolotls, transferred to 4 aquariums, 3 in each. In the aquariums the animals found themselves under conditions of constant temperature and lighting and the same amount of water. In all the aquariums thyroidin was added in dilution, the concentration being 0.01 g/l in two of the series and 0.075 g/l in one. In one of the aquariums sodium bromide was added in addition to thyroidin (0.05% solution of NaBr), in another, sodium iodide (0.05% solution of NaI), and in a third, both sodium bromide and sodium iodide, each salt in a concentration of 0.025% so that together the total concentration of the two salts equalled 0.05%. The animals in the fourth aquarium, in which only thyroidin was dissolved, served as the control.

During the process of metamorphosis each axolotl was systematically noted for weight, gill length, width of tail, color, and time when it crawled out into dry land and transferred to lung breathing. These data were able to illustrate one or another effect of each of the haloids on the dynamics and rapidity of the metamorphosis.

EXPERIMENTAL RESULTS

In all three series of experiments we obtained identical results. The most characteristic trials are presented in the graphs in Fig. 1, showing the dynamics of the changes (per day) in the gill length, width of the caudal fin, and weight of the animals.

In the control animals that were only subjected to the effects of thyroidin only, minimal changes were observed during the first 20-25 days in the gill length, width of the tail, and weight; on the curve this is manifested in the form of a peculiar "plateau", most clearly expressed in relation to the changes in gill length (see Fig. 1, A). Following this "plateau", the curves trace a marked shift for all the criteria noted above, and on the 35-50th day the metamorphosis of the axolotl converts it from the larval to the adult form – the amblystoma.

In the experiments on the effect of sodium bromide on the axolotls in addition to thyroidin, the metamorphosis ran a different course. The "plateau" which was

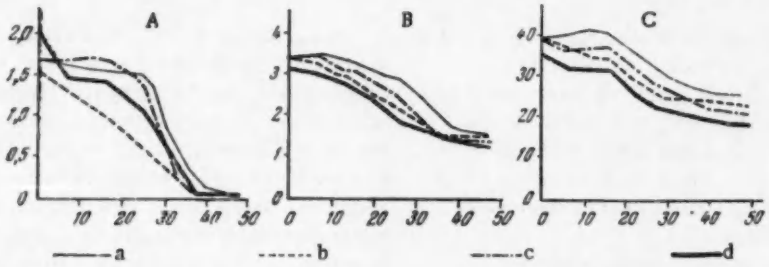

Fig. 1. Changes in the gill length (A), width of the caudal fin (B), and weight (C) of the individual axolotls under the influence of thyroidin (control), and the simultaneous effects of the salts of iodine and bromine. Concentration of thyroidin 1:100,000. On the ordinate axis – gill length in centimeters (A); height of the caudal fin in centimeters (B); weight of the axolotl in grams (C); on the abscissa axis – duration of the metamorphosis (in days); a) Control; b) NaBr; c) NaI; d) NaBr, NaI.

observed in the control animals here either completely disappeared or remained poorly noted. Thus, the effect of bromine appears to be mainly on the first stages of the metamorphosis.

Under the conditions testing the effect of sodium iodide, the "plateau", although also decreasing, did so very minimally in comparison with that which was observed in the control experiments; in addition, in several experiments there was even noted an increase in the gill length and other indicators during the first days of the metamorphosis.

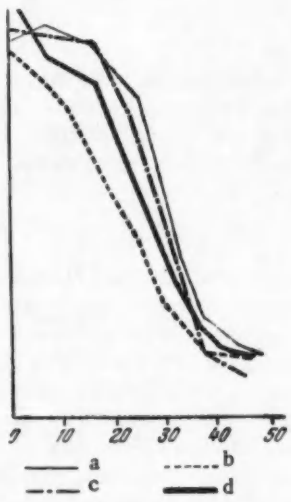

Fig. 2. The general "metamorphosis curve", occuring under different conditions, according to the criteria of gill length, height of the caudal fin, and weight of the animal. Symbols are the same as in Fig. 1.

The experiments with the simultaneous actions of the salts of bromine and iodine on the metamorphosis of the axolotls also showed a disappearance of the "plateau" observed in the control animals, although here this phenomenon was manifested to a somewhat lesser degree than in the experiments with the action of bromine alone.

If, on the basis of all the criteria of metamorphosis selected by us (changes in the gill length, width of the caudal fin, and weight), one general curve, arbitrarily called the "metamorphosis curve", is traced for each animal, then it is possible to clearly see the inferences noted above (Fig. 2).

At a concentration of 0.01 g/l of thyroidin the metamorphosis of the axolotls exposed to the action of either the bromine or iodine salts, as well as to their joint activity, was completed 5-10 days earlier than in the control animals (Fig. 3).

At higher concentrations of thyroidin (0.075 g/l) this difference was less manifest. On the average, the duration of metamorphosis of the control axolotls was greater than the duration of metamorphosis of the axolotls subjected to the action of haloids by 3-7 days (see table).

Statistical analysis of the material showed the obtained difference to be significant. We were not successful in detecting a significant difference in the rapidity of the metamorphosis within a comparison of the data from the experiments with bromine, iodine, and their conjoint activity.

Following the completion of metamorphosis several of the animals died. The largest number of axolotls died in the experiments with the action of the iodine salt (11 animals), a smaller number in association with the action of the bromine salt (8 animals), still fewer with the conjoint action of the salts of bromine and iodine (5 animals), and the fewest – in the control experiments (3 animals).

From this one may postulate that iodine possesses a greater toxicity than bromine. The simultaneous

action of the bromine and iodine salts probably, to some degree, neutralized their toxicity, although their total concentration, as we already noted, was the same as in the experiments using either one of the salts. One can consider that bromine and iodine act on different phases of the metabolism, and, thus, their joined effect was less toxic than in those cases where either one of the haloids acted alone.

As is well known, iodine is necessary for the construction of the active portion of the thyroid gland hormone – thyroxin – and as far as this is concemed the mechanism of action of iodine is more or less clear. In regard to bromine, here great investigative work is still needed for a concrete answer.

According to the data in the literature [4,7], microdoses of bromine show a stimulatory effect on the thyroid gland, manifested by its hyperplasia, augmentation of the secretory activity of the epithelium, and the accumulation of colloid within it. It is interesting that the thyroid gland of the animals subjected to the action of bromine shows a stronger influence on the metamorphosis of the amphibia than the gland of the intact animals [7]. At the same time, basal metabolism, under the influence of bromine, either does not change or decreases. In addition, the accumulation of iodine by the thyroid gland is shown to be considerably decreased [4].

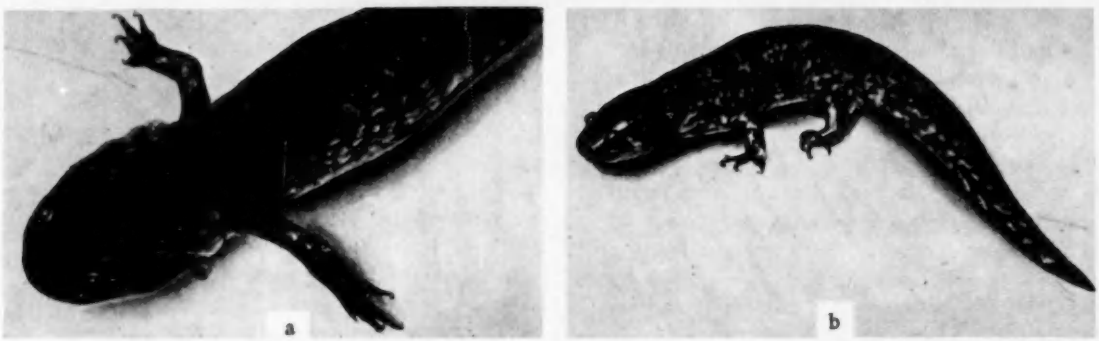

Fig. 3. Conversion of the axolotl to an amblystoma with the action of thyroidin (a) and the simultaneous action of thyroidin and sodium bromide (b) on the 49th day of metamorphosis.

The experimental material of the present work and the data in the literature compel postulation that bromine derivatives of the thyroid gland stimulate the metamorphogenic conversion of the so-called mucigenous protein to a more highly differentiated "dry protein", that being graphically displayed in the metamorphosis of the amphibia although the same process, obviously, occurs in all vertebrate animals.

Thus, bromine may be regarded as a synergist of iodine in its participation in the process of metamorphogenic conversion of the protein, and as a relative antagonist to the accumulation of iodine in the thyroid gland and in its participation in the processes of basal metabolism. On the strength of the close chemical relationship of bromine and iodine, the former can, in a number of instances, take the place of iodine in the thyroxin molecule or the amino acids participating in its structure. It is known that compounds such as these are encountered in nature [15,18], and may be obtained artificially [21].

Thus, it may be postulated that one of the means of action of bromine on the organism via the thyroid gland is the replacement of the atoms of iodine in thyroxin by atoms of bromine, so that the effectiveness of the hormone's action, and, possibly, even the character of its action, is determined by the number of replaced atoms.

SUMMARY

The author compared the effect of bromine and iodine on metamorphosis of Amblystoma mexicanum. 52 axolotls were subjected to the action of thyroidin (0.01) and haloid salts: 0.05% NaI solution in the second, 0.025% NaBr and 0.25% NaI in the third and fourth series of experiments. Apart from thyroidin no other additional salts were introduced.

As shown, during thyroidin action bromine and iodine salts tend to accelerate the metamorphosis of A. mexicanum, by 5-10 days. Bromides exert a higher effect on the primary metamorphosis stages. Because of these, the dynamics of metamorphosis under the effect of bromides differs from that under the action of iodides. A suggestion is made that brimine participates in the thyroid gland function.

Control	0.05% NaBr	0.05% NaI	0.025% NaBr 0.025% NaI
Thyroidin in a concentration of 0.01 g/l			
50	30	35	40
50	30	40	35
40	45	40	45
40	35	35	35
45	40	40	35
50	30	42	30
50	40	40	40
50	45	45	30
Thyroidin in a concentration of 0.75 g/l			
35	30	30	32
32	30	30	35
35	32	30	35
35	30	32	35
35	30	32	35
Total average value			
42±2,2	34±1,8	36±1,7	36±1,5
Average for the experiments with thyroidin in a concentration of 0.01 g/l			
47±1,8	37±2,5	40±1,5	36±2,0

Rapidity of the Metamorphosis of Axolotls Under the Influence of Thyroidin and Salts of Bromine and Iodine (in days)

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DYNAMICS OF THE MITOTIC ACTIVITY IN CORNEAL EPITHELIUM OF WHITE MICE DURING FASTING

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The study of the interrelationship between metabolic processes and cellular multiplication is at the present time one of the important ways of exposing the mechanisms regulating cellular division. From this point of view, the study of the cellular multiplication process during the action on the organism of factors which significantly change or disturb the dynamics of metabolic processes becomes expeditious.

By utilizing the generally known fact [4] concering the lowering of the metabolic level during fasting, the authors decided to utilize this occurrence as a methodical procedure for the study of the dependence of cellular multiplication on the intensity of metabolic processes in the organism.

A number of authors [5, 6, 7] who have investigated the mitotic activity of skin epidermis, intestinal epithelium and of the liver from white mice and rats, have noticed a lowering in the number of mitoses during fasting. However, the majority of the investigators in these experiments did not encounter an especially high sensitivity of the cells toward various changes in the surrounding medium, among which were changes connected with the experiment itself. The latter could basically influence the mitotic regimen. This circumstance can be ascertained only when a sufficiently large number of control animals is introduced into the experiment. It is necessary to note that up to the present time the dynamics of mitotic activity during the fasting process, have not been sufficiently studied. Authors of a large number of similar experiments did not concern themselves with the role of metabolism in the concurrently occurring changes. The mitotic activity of such a convenient study object as the corneal epithelium of mammals has actually been subjected to an investigation during fasting. Only partial data [1,2] have been obtained on several animals.

Previously completed investigation [3] concerning the dynamics of mitotic activity in corneal epithelium of fasting young rats has shown that mitotic activity became lowered only on the third day and was dependent on the degree of the animal's exhaustion. A supposition was expressed that changes in mitotic activity during fasting were connected to the degree of exhaustion of the organism's energy resources.

Taking into account that mice have a higher basal metabolism than rats, the author decided to investigate the dynamics of mitotic activity in corneal epithelium during fasting of these animals.

In order to clarify some links in the mechanism responsible for the change in mitotic activity during fasting, an attempt was made to compensate the energy losses of the fasting animals by giving them carbohydrates.

EXPERIMENTAL METHODS

The experiments were conducted on 105 sexually mature male white mice with an average weight of 20 g. For the experiment the animals were held for 5-10 days under standard conditions with 5 animals per group. For each experimental group there was a corresponding control group.

In the first series, the experiment animals, as opposed to the controls, were not given feed and were given only water.

In the second series, besides the control group and the fasting group, there was a group of mice receiving unlimited amounts of carbohydrates (20% solution of sugar and small pieces of sugar or 40% glucose). The feeding and watering of the mice was conducted at the same time in all experiments. The animals were killed after 1, 2, 3, 4, or 6 days of fasting. Mitoses were counted in total histological preparations of the cornea, in its epithelial layer, with a consequent counting of the number of mitoses in 100 visual fields, corresponding to $1 mm^2$ of its area.

EXPERIMENTAL RESULTS

Average number of mitotic indices of the first experimental series, according to each interval of complete fasting, are presented in Table 1.

As is evident from the data in Table 1, fasting for the period of one day did not produce a lowering of mitotic activity in the corneal epithelium of the experimental animals. In fasted mice during this fasting interval, there

TABLE 1. Mitotic Activity of Corneal Epithelium From Fasting White Mice

Control mice				Fasting mice			Remarks
duration of the experiment (in days)	number of mice	mitotic index	weight changes (in percent of the original)	No. of mice	mitotic index	wt. changes (percent of original)	
1	5	338	+3	5	409	—10	
2	5	305	Without change	5	49	—22	
	5	456	" "	5	36	—25	
3	5	463	+6	4	45	—28	Toward the end of the experiment one died
4	5	218	Without change	2	60	—34	During the experiment 3 mice perished
6	5	300	" "	2	41	—35	The same

was observed even an increase in the mitotic number (to be truthful, it was statistically insignificant), in a way similar to the observation in rats after two days of fasting [3]. The weight of experimental mice decreased by 10%.

Fasting of mice for the period of two days, which was accompanied by weight loss of 25%, led to a sharp lowering in the mitotic activity of the corneal epithelium (6-12-fold) in comparison with the control (P = 0.000). In animals fasted for three days, there was a 28% weight loss. Mitotic activity of experimental animals was 10 times lower than in the control group (P = 0.0000). From among five mice, one perished toward the end of the experiment. During fasting for four days only two out of five experimental animals survived. The weight of the fasted animals decreased by 34%. Mitotic activity of fasted mice appeared considerably lower than in fed mice (P = 0.0000). With a more prolonged fasting period (6 days), only 20% of the experimental mice survived (in this experiment 10 animals were fasted, 5 in each group). Weight loss of these mice comprised 35%, mitotic activity was decreased almost 8-fold (P = 0.02).

The sharp decrease of mitotic activity in the experimental mice of the first series, which was accompanied by a large weight loss during the fasting, could be explained first of all by an insufficiency of energy materials for the processes of cellular proliferation. A second series of experiments was conducted in order to clarify the above question; energy expenditures of the fasting mice were restored by feeding them sugar or glucose. Results of these experiments (mean mitotic indices and weight changes of the animals) are presented in Table 2.

From the data presented in Table 2 it is evident that in fasted mice the mitotic index after two days of the experiment was lowered by more than 6-fold, while at the same time in sugar-fed mice the number of mitoses in the corneal epithelium was not different from the controls (the difference was not statistically significant (P=0.845).

The fact that animals receiving sugar lost only 5% of weight when the fasted lost 20% deserves attention. Supplemental feeding with carbohydrates alone for four days (mice were given a 40% glucose solution) left the mitotic activity practically at the level characteristic for control animals (difference statistically not significant; P = 0.565).

At the same time, of the five fasted mice receiving only water, two were left alive; the mitotic activity was lowered 4-fold.

The data for mitotic activity are in agreement with the data for weight loss; mice receiving glucose lost 8.6%, mice receiving only water, 34% of weight.

TABLE 2. Mitotic Activity of Corneal Epithelium of Fasting and Carbohydrate-Fed Mice

Length of the fasting interval (in days)	Control			Full fasting			Sugar feeding		
	number of mice	mitotic index	weight change (in percent of initial mouse weight)	number of mice	mitotic index	weight change (in percent of initial mouse weight)	number of mice	mitotic index	weight change (in percent of initial weight)
2	5	358	Without change	5	54	20	10	347	5
4	5	220	" "	2	63	34	5	191	8.6
5	5	249	" "	5	All died		5	148	17

Extension of the glucose-only (40% solution) feeding period to five days (inclusive) resulted in pronounced lowering of the mitotic activity ($P = 0.004$). The weight of mice receiving glucose dropped by 17%.

The obtained data indicate that in mice mitotic activity was lowered earlier (on the second day) than in rats (on the third day).

This could be explained by the fact that in mice, which are distinguished by a high level of basal metabolism, during fasting there takes place rapid utilization of energy stores. In this case the compensation of energy stores occurs at the expense of protein destruction [4], and is evidently the final cause for the pronounced lowering of mitotic activity in the corneal epithelium of fasting animals. It should be noted, however, that even during the stage of pronounced exhaustion produced by the fasting, when the animals lost 25-35% of the initial weight, mitoses did not disappear completely.

Materials of the experiments where the fasting mice were fed carbohydrates gave basis for supposing, that during compensation of the organism's energy stores by carbohydrates alone, the mitotic activity of the corneal epithelium was supported at the normal level for four days of fasting inclusively. This is evidently explained by the presence of a sufficient amount of flexible materials in the organism which, while not being utilized for energy needs during fasting, make possible the processes of physiological regeneration at a level close to normal. However, after five days of fasting and in glucose-fed mice there occurred a pronounced lowering of mitotic activity. This lowering, evidently, occurs due to a disturbance, in the flexible-materials metabolism, produced by protein insufficiency.

SUMMARY

The dynamics of mitotic activity of corneal epithelium was studied in fasting white mice. The animals were deprived of food for a period of 1 to 6 days. A sharp decrease of mitotic activity, appearing from the second day of fasting, remained on the same level with more prolonged food deprivation as well. In mice which received only carbohydrates (sugar and glucose) the mitotic activity of the corneal epithelium remained normal up to 4 days inclusively; after the fifth day of experiment, however, the mitotic activity decreased. The loss of weight in mice given water only was four times greater then in those fed on carbohydrates.

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INNERVATION OF RABBIT VIRAL PAPILLOMA

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In studying the neural mechanisms involved in tumor development, morphological analysis of the course of reflexive and trophic influences of the nervous system on this process is of importance. It may be assumed that the influence of the central nervous system is transmitted to malignant neoplasm directly via the peripheral nervous system, or indirectly via some part of the humoral system. However, the problem of which of these pathways is the most important is at present unsolved. The obvious approach is to ascertain whether or not there is a direct neural pathway connecting the tumor with the central nervous system. The majority of investigated tumors, both human and animal, have been found to have their own innervation [2,5-9, etc.]. In the available literature, we did not find any similar investigation of viral malignant papilloma in rabbits.

Considerable attention for some time has been devoted to the virus as an etiological factor in the formation of pathological tumors. Investigations of the present type, therefore, are of great interest.

METHODS

Experiments were carried out on 32 rabbits. Papillomas were produced by smearing virus material onto scarified skins. The Campos method was used for staining nerves. Papilloma innervation was studied at various post-inoculation intervals: 10 days, 1,2,5, 7, 8, 14, 16 months. Papilloma malignization usually set in at the 10-12th month. With these procedures we studied the innervation during both the benign and malignant stages.

RESULTS

Ten days after virus inoculation, microscopy revealed considerable hypertrophy of cutaneous epithelium and epithelium of hair bulbs. Neural elements at this time were found in considerable quantity. The majority of nerve fibers, with various calibres from very thick to very fine fibrils, are located in the primary connective tissues. In some preparations, fine branches can be seen to leave the principal fibers and travel out to areas between hair bulbs. Some of these fibers entwine the hair bulbs, and single fibers may penetrate the epithelial lining of the hair sac. Fine nerve fibers, devoid of myelin sheath, penetrate the cutaneous epithelium to form tightly coiled receptor structures. The morphology of these fibers appeared normal (Fig. 1).

At 1 month after virus inoculation, small individual papillomas, 0.2 x 0.2 cm in size, were present in the skin. Study of these growths also revealed nerve fibers located chiefly in the connective tissue stroma beneath the papilloma. Individual bundles of fibers travel out to the basal layer of papilloma epithelium. We were not successful, however, in finding any branches of these fibers in the overlying epithelial growth. These nerve elements appeared normal. It should be pointed out that satisfactory staining of nerve fibers at 10 days or 1 month following virus inoculation was difficult to achieve; neural elements in the tissue often could not be exposed.

Study of neoplasm at 2 months after virus inoculation revealed a distinct transition of hair bulb epithelium. The papilloma at this time shows a uniform basal layer submerged in subcutaneous tissue. Its peak is covered with a small horny mass and is elevated above the skin. Great numbers of nerve bundles, composed of fibers of various calibres, are found in the underlying connective tissue. Some of these fibers form delicate single nerve endings. Individual fibers, running in subcutaneous tissue, penetrate the papilloma epithelium a considerable distance. In some areas preserved coils of nerve fibers around hair bulbs can be noted. The very fine fibers forming these coils show reactive changes (vacuoles, axolysis, swelling) along their course.

At 5 months after virus inoculation papilloma had attained considerable size, and their surfaces were covered with an extensive horny mass. These growths

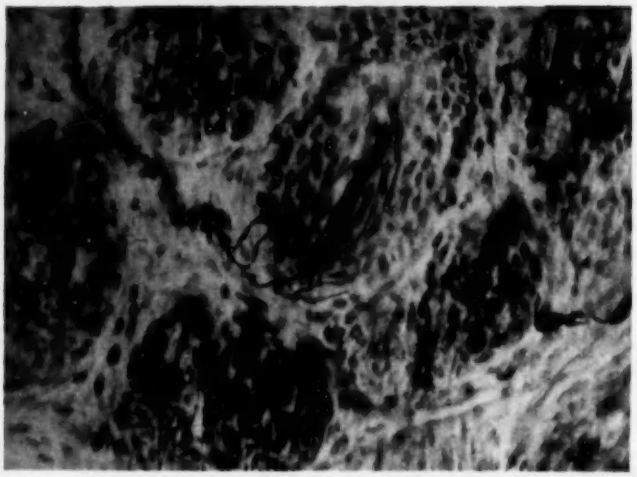

Fig. 1. Innervation of hypertrophied hair bulbs 10 days after virus inoculation. Objective, 32×; ocular, 7×; coefficient, 1.4. Campos stain.

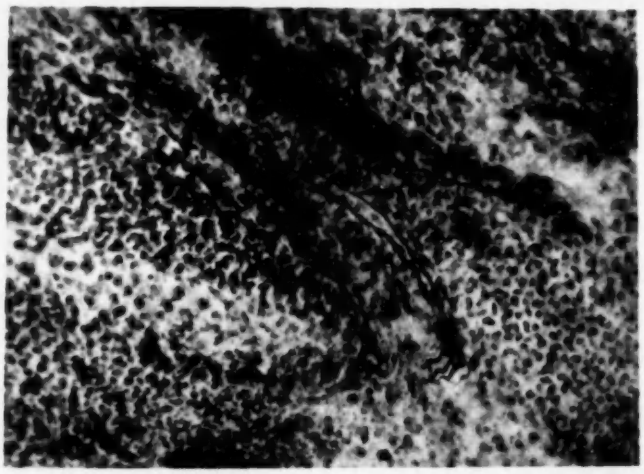

Fig. 2. Penetration of nerve fibers into papilloma at 5 months after virus inoculation. Objective, 32×; ocular, 7×; coefficient, 1.4. Campos stain.

were circumscribed by primary connective tissue, into which the epithelial protuberance was deeply wedged. Considerable evidence of mitosis was observed, particularly at the level of the 4-5th layers. The primary mass of nerve fibers is located in the underlying connective tissue, and bundles of nerve fibers travel into the connective tissue stroma of the epithelial protuberance.

The nerve fibers, penetrating the stratified squamous papilloma epithelium with equal density, are fine, straight, and unarborized, but at the same time irregularly thickened (Fig. 2). Many of these dichotomize and form more intensively argentophilic swellings. Some of the more delicate coiled fibers form club-shaped endings similar to those of receptor terminals. Many of the fibers accompany vessels and branch into their walls. In some places itis possible to observe individual fibers sending one branch to vessel walls and another to epithelial cells.

At 7 months after virus inoculation the morphological structure of the papilloma agreed with the usual description. The large epithelial growth is deeply wedged in the surrounding connective tissue, and the latter clearly circumscribes the basal layer of epithelium. The epithelial protuberance has expanded to a form of papillo-acanthosis. Evidence of extensive mitosis is visible. Microscopy of these papillomata reveals that nerve bundles containing

3-4 fibers or more leave the connective tissue and penetrate a considerable distance into the stratified squamous papilloma epithelium. These fibers are uniform, smooth, and devoid of any pathological changes. Neural structures similar to receptors are found both in the connective tissue stroma of the epithelial growth and in the surrounding connective tissue. In the latter a great number of vessels are seen. In some areas the nerve fibers are vacuolized and irregularly thickened due to irritation.

The neural elements present approximately the same morphological picture at 8 months after virus inoculation. In this case also the nerve fibers penetrate the stratified squamous papilloma epithelium. These are fine, delicately stained fibers, whose characteristics indicate that they grew into the papilloma and were not located there previously. Some of these fibers give off fine branches with bead-shaped varicose enlargements typical of growing axons. In the surrounding connective tissue the nerve fibers form fine branched endings similar to recepters. Club development can be noted on individual fiber terminals. In this case, therefore, there is the appearance of stimulation and development of the nerve fibers of papillomatous tissue.

These characteristics differed from those observed during the benign period of papilloma growth. Studies of even later stages of the tumor process, i.e., at the beginning of malignization or after the complete transformation of the papilloma into a malignant tumor, revealed more conspicuous changes in nerve fiber structure. Such a picture was observed at 14 months after virus inoculation, i.e., at the initial stage of the malignant process. Randomly located islands of stratified squamous epithelium occur in the loose connective tissue. Their centers contain a mass of keratosic epithelium. Together with these changes occurs a region of preserved normal papilloma structure. Nerve fibers of various diameters can be noted among the stroma collagen fibers, and between stroma epithelial cells. The majority of nerve fibers however, are fine, irregularly thickened, and hyperargentophilic. In some cases, thickened nerve fibers containing vacuoles can be seen.

Sometimes nerve fibers, running in primary epithelial tissue, reach almost up to the surface of the tumor. It was our impression that the apex of the nerve bundle occurs in the peripheral zone of the tumor and is exposed to destruction. The nerve fibers in this region undergo degenerative changes. The large myelinated fibers are severely thickened and have become tightly coiled. They form a rough curve, and turn to travel back towards the base of the tumor, gradually thinning out. From the thickened parts of fibers emerge very fine collaterals with club-shaped endings, and these collaterals sometimes show a retrograde course into the base of the tumor. Parts of the thick fibers are exposed to complete destruction into clumps. In the main bundle, where nerve fibers are loosely arrayed, secondary protuberances are also prominent, emerging both from fine and thick nerve trunks which exhibit signs of pathological excessive growth of neural elements. In some cases, nerve fibers adjacent to epithelial cells are abruptly twisted, with considerable irregularity in diameter and thorniness of contours. The endings of very fine nerve fibers are conspicuously thickened. In the depth of the tumor, where there is a mass of connective tissue, the fibers are ordinary, with uniform contours and small swellings on the endings.

Study of tumors at 16 months after virus inoculation, i.e., when the papillomata had made a complete transition to the malignant stage, revealed more conspicuous degeneration of neural elements. At this stage the tumor is deeply ingrown into the mass of muscle. It can be seen that some nerve fibers found in the connective tissue stroma are irregularly swollen and highly argentophilic. Single nerve fibers which leave the main bundle to form structures very similar to receptors can occasionally be seen. These structures occur in the connective tissue layers between epithelial cells. The nerve fibers have conspicuous swellings which produce secondary protuberances, and these undergo degenerative destruction. Axolysis of axis cylinders is observed in the thickened regions. Fine terminal rings lie scattered in the tissue. In the loose connective tissue in the depth of the tumor are found bundles of nerve fibers in various stages of degeneration (Fig. 3).

A great many nerve fibers running in bundles occur in the connective tissue stroma among epithelial cells. Some of these fibers also show signs of destruction and excessive growth (conspicuous swelling along the length of the fiber, degenerating secondary growths). It can be seen how the fibers, running in bundles, vary their character when in contact with tumorous tissue. Here the nerve fibers abruptly thin out. Individual fibers, emerging from bundles, travel towards the epithelial cells.

These experiments showed that nerve fibers occur in both the benign and malignant developmental stages of viral papilloma. On the 10th day after virus inoculation, when only negligible proliferation of cutaneous and hair bulb epithelium had occurred, normal neural elements could be observed in the latter. A similar picture was also noted at 1-2 months after virus inoculation, i.e., after the pathological process had already commenced.

The first reactive change of nerve fibers was noted at 2 months after virus injection. With development of the pathological process, this change was increased. Even if these changes were reversible during the benign stage of papilloma formation, with transition to the malignant stage profound degeneration of neural elements was noted.

Along with nerve fiber degeneration, excessive growth was observed, and this, undoubtedly, is evidence of a reaction of nervous tissue to pathological stimulation, leading to nerve growth, in the later period of tumor development

Fig. 3. Large nerve trunk located deep in tumoral tissue. Degenerative and reactive changes in nerve fibers are visible. Microphoto of tumor at 14 months after virus injection. Objective, 32×; ocular, 6×; coefficient, 1.9. Campos stain.

(5-8 months, etc.), into various sections of the neoplasm. Innervation consisting of nerve fiber growth into the connective tissue capsule of the tumor is particularly evident.

SUMMARY

Innervation of viral papilloma was studied in rabbits. The nerves were stained by the Campos method. The viral papilloma of rabbits was found to be innervated. The nerve elements were revealed in 10 days, 1, 2, 5, 7, 14, and 16 months after the inoculation of papilloma virus. The nervous formations observed at the early dates after the virus inoculation could be considered as normal; later, an active growth of the nerve elements occured, manifested both in the changed character of the nerve fibers themselves, and in the appearance of growth bulbs at their endings.

Reactive and degenerative changes were also seen in these nerve elements, the extent of which increased with the development of the viral papilloma, especially after its malignization.

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CHANGES IN SUBCUTANEOUS CONNECTIVE TISSUE OF RATS AFTER INJECTION OF CORTISONE

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During the past 10 years there have been obtained numerous data concerning the potent antiinflammatory action of cortisone. It was shown that cortisone retarded such components of the inflammatory process as vascular dilation, exudation, marginal standing of leucocytes, fibroblast proliferation and the development of granular tissue [7, 11, 13, 14, 15, 18, 19, 20, 21]. The antiinflammatory properties of cortisone have promoted its wide utilization in clinical practice.

Considerably less work has been devoted to the study of the cortisone action on connective tissue found under normal conditions. Investigation in this direction touched mainly upon the action of cortisone on the ground substance and on the fat cells of the connective tissue. A number of authors have presented results concerning the influence of cortisone on the lowering of hyaluronic acid on the ground substance of connective tissue [8, 9, 12, 16] and on the decrease in number and degranulation of fat cells [8, 9, 10, 12, 17].

The data are quite scanty concerning the action of cortisone on other cellular components of normal connective tissue. Of interest are the investigations of I. A. Alov [1], who observed that under the influence of cortisone there were stimulations of the fibroblast differentiation process and of formation of the ground substance in subcutaneous connective tissue of mice.

The problem of this study is to investigate the action of cortisone on various components of rat subcutaneous connective tissue and to establish the character of the changes produced by this hormone.

EXPERIMENTAL METHODS

Rat subcutaneous connective tissue was studied in film preparations, prepared by the method of G. Yasvoin For a more accurate evaluation of changes in the cellular composition of connective tissue there were counted the percentage ratios among various cellular elements [3-6]. The following cellular forms were differentiated: 1) young fibroblasts with one or two offshoots; 2) mature fibroblasts with a clearly defined ectoplasmic layer and numerous offshoots; 3) fibrocytes– differentiated fibroblasts with strongly elongated and sometimes deformed nucleus and sharply reduced cytoplasm; 4) macrophages; 5) lymphoid cells; 6) polymorphonuclear leucocytes; 7) degenerating deformed cells, whose origin was difficult to ascertain. One thousand cells were counted in each preparation.

The author has also utilized various histochemical methods for the study of connective tissue: vital staining with neutral red; staining with Sudan black for lipids; staining with toluidine blue with phosphotungstic acid fixation [2] for ground substance; staining with toluidine blue after fixation for chromotropic mucopolysaccharides; staining according to van Geison for collagen; reaction with periodate fuchsin sulfurous acid (PAS reaction) for polysaccharides.

The experiments were conducted on rats weighing 50-60g; Cortisone was injected intraperitonially. In the first series of experiments, two groups of rats (5 rats per group) were injected one time with doses of 5 and 12.5 mg, in the control experiments, 3 rats were injected with 0.5 ml of physiological solution. The animals were killed with ether on the following day.

In the second experimental series rats were injected with 12.5 mg of cortisone for a day (two injections per day); a control group of 3 rats was given physiological solution. The animals were killed five days after the last injection. From subcutaneous connective tissue of each rat's back there were prepared three film preparations and the cytograms were counted.

EXPERIMENTAL RESULTS

In the table are presented data obtained in the experiments.

As is evident from the table, the percent of fibrocytes in the subcutaneous connective tissue increased sharply after a single injection of cortisone. With respect to other cells, particular changes in comparison with the control were not noticed. There were no differences in experimental results from a single injection of 5 and 12.5 mg of cortisone.

Repeated cortisone injection produced a more clearly expressed action on connective tissue. Leucocytes and

Changes in the Percentage Relation of the Cellular Composition of Rat Subcutaneous Connective Tissue Following Single and Repeated Injections of Cortisone

Connective tissue cells	Control	Experiment		
		single injection		repeated injection
		5 mg	12.5 mg	12.5 mg
	6 rats	5 rats 12.5 mg	5 rats	4 rats
Polynuclear cells	0,51±0,17	0,36±0,18	0,66±0,25	—
Lymphoid cells	1 45±0,19	0,73±0,05	1,04±0,1	0,8±0,04
Macrophages	26,95±4,5	18,16±0,59	16,92±0,98	13,83±0,16
Young fibroblasts	1,36±0,25	1,03±0,52	2,02±0,66	—
Mature fibroblasts	69,3±4,49	71,1 ±0,52	78,58±1,59	85,27±0,16
Fibrocytes, within the above number.	9,93±2,36	40,6 ±11,67	39,1 ±8,08	65,27±4,03
Degenerating cells	0,41±0,15	3,6 ±0,7	0,78±0,19	0,1 ±0,04

young fibroblasts disappeared. The percent of fibrocytes increased even more; many of the cells belonging to this group had a sharply deformed pycnotic nucleus, strongly reduced cytoplasm, and resembled degenerating cells. The cytoplasm of many mature fibroblasts contained large vacuoles. Reduced cytoplasm was encountered in many macrophages.

In the third series of experiments we studied, by means of histochemical methods, the action of repeated cortisone injection on the connective tissue. Cortisone injection and preparation of connective tissue films was done by the same method as in the second experimental series. Thirty-six rats, divided into six equal groups, were used in the experiments; three rats from each group served as controls. Connective tissue films were stained with one of the previously described histochemical methods; control films were stained together with the experimental.

In the case of vital staining with neutral red, in the residual cytoplasm of the fibrocytes, especially numerous in experimental animals, there was noted a small number of fine red granules. Macrophages contained a considerable number of more coarse granules. In the control and in the experimental there were not encountered any diffusely stained cells.

After staining with Sudan black the cytoplasm of control and experimental polynuclear cells was filled with fine lipid granules, quite monotypic in diameter; in the fibroblasts the endoplasm was stained, whereupon more intensely stained portions were situated closer to the nucleus. The nuclei were not stained. The cytoplasm of control and experimental fibrocytes was stained more intensely than the cytoplasm of fibroblasts. In the connective tissue cells of control and experimental animals granular fat deposits were not observed.

With other staining methods (according to van Gieson; toluidine blue with phosphotungstic acid fixation; toluidine blue for metachromatic compounds; periodate fuchsin sulfurous acid for polysaccharides) there could not be established any changes after cortisone injection as compared with the control.

As is evident from the obtained data, the increase in the number of fibrocytes was most characteristic of the action of cortisone on the subcutaneous connective tissue of the rat. Young monoplasmic fibroblasts disappeared from subcutaneous connective tissue after repeated injection of cortisone; together with this there was an increase in the number of highly differentiated fibroblasts. These data are in agreement with the results of I. A. Alov's investigations on mice [1]. During the differentiation process the fibroblast goes through the stage of a diplasmotic vacuolar cell and into a fibrocytic, with a sharply reduced cytoplasm and a deformed nucleus. However, as follows from the present experiments, cortisone did not produce cellular injury, because even fibroblasts in the last stages of differentiation did not show signs of degeneration.

I. A. Alov assumes that the process of intensified fibroblastic differentiation is accompanied by the formation of ground substance. This takes place, as he assumes, at the expense of fibroblastic ectoplasm dissolution by growing vacuoles or via fragmentation of ectoplasmic offshoots. During the action of cortisone there were not observed any increase in the quantity or changes in the morphological peculiarities of the ground substance. Staining for collagen, mucoids and ground substance did not reveal any differences in connective tissue from control and experimental animals, consequently, the process of fibroblast differentiation from a mature fibroblast to a fibrocyte was not accompanied by an increase in the amount of ground substance. The formation of the latter, evidently, is the function of the young cells of the fibroblastic series.

It is of interest to compare the present results with the data of V. V. Shikhodyrov [6], who, by subjecting dogs to x-ray irradiation, has also obtained stimulation of the fibroblast differentiation process in subcutaneous connective tissue. In the given case x-rays produced an effect on connective tissue similar to the influence of cortisone.

SUMMARY

The author studied the effect of cortisone on the relative quantities of different cell forms on the histochemical characteristics of cells and of ground substance in the subcutaneous tissue of normal rats. Cortisone stimulated the process of fibroblast differentiation, causing a striking increase in the mean percentage of the most differentiated fibroblasts (fibrocytes). Cortisone provoked no degeneration of fibroblasts or any changes of histochemical peculiarities in the ground substance or in the connective tissue cells. No increase in the amount of the ground substance was noted during differentiation of fibroblasts into fibrocytes.

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EXPERIMENTAL INFLAMMATION OF THE HEMOCHORIAL PLACENTAE

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It is known that inflammatory placental changes are the reason for many forms of gynecological pathology. Pathomorphological changes in the placenta in the form of acute or chronic inflammations are observed during maternal infectious diseases: pregnancy toxemias, heart imperfection, kidney diseases, etc. There are indications that in these cases there are considerable changes in trophoblastic enzyme systems [6, 8] and in the permeability of the connective tissue of the fetal placenta [2]. All of the above data were obtained during investigations of the placenta at the height of the operation and do not give presentations concerning the peculiarities of inflammatory reaction in placental tissues.

Thus it appeared expedient to study placental inflammation in an experiment on animals with hemochorial type of placenta. During the past few years attempts have been made in this direction [3, 5, 7], but the results were contradictoryin many respects and did not give the systematic characteristics of the inflammatory reaction of placental tissues.

EXPERIMENTAL METHODS

White rats were utilized in the experiments for the study of the placental inflammatory reaction. The inflammation was produced by introducing a foreign body into the placenta of 16-18 day embryos. For this purpose, the placenta together with the uterus were pierced with a silk thread which was left in the traumatized portion; this also accomplished the fixation of the placental disk. Altogether, 25 animals were operated on. The placenta was studied in 12,24 hours and 2,3 and 4 days after introduction of the foreign body. The animals were killed by bleeding, after which the placenta, with the corresponding part of the uterus, was fixed in 12% formalin. Serial paraffin sections were stained with Ehrlich's hematoxylin-eosin azure 2-eosin and picrofuchsin. The inflammatory reaction developed differently in the fetal and maternal portions of the placenta. For this reason it is expedient to present separately the results of the investigation on the labyrinthic and extralabyrinthic portion of the placenta.

EXPERIMENTAL RESULTS

Inflammatory reaction of the extralabyrinthic portion of the placenta. During the first 12 hours after introduction of the foreign body the surrounding tissues were subjected to destruction. Wide portions of the cytotrophoblast, during this stage, were represented by a structureless basophilic mass. Among the disintegrated and changed elements there were encountered individual surviving cells and even cells in mitotic division. The whole marginal zone was infiltrated with special leucocytes. In portions bordering with the marginal zone there was observed clearly expressed hyperemia, which was accompanied by hemorrhages and infiltration by leucocytes of the cytotrophoblast of this region. Proliferation in this region was expressed very weakly; infrequently in the cytotrophobastic elements there were encountered amitosis and binucleated cells. Mitosis was absent.

At the end of the first day the marginal zone was still represented by changed cytotrophoblastic elements. Their cytoplasm was poorly stained and strongly vacuolated; the nuclei were shrunken and deformed. In many cells the nuclei were in the state of caryorexis or were completely absent. In the region of extravasation there could be observed a large number of round or polygonal basophilic cells with a vacuolated protoplasm and compact nucleus, situated predominantly eccentrically; their cytoplasm there were encountered phagocytized erythrocytic fragements. The topography, relation to stains,and morphology of these elements permitted one to consider them as transformed decidual cells, which under inflammatory conditions acquired phagocytic ability. The marginal zone was abundantly infiltrated with leucocytes, prodominantly of the special variety, with strongly segmented nuclei. Among the necrotic cytotrophoblostic elements there were encountered large cells with peculiar nuclei; instead of the large nuclei characteristic for these cells,in the cytoplasm was situated a group of small nuclei, surrounded by a thin common membrane (Fig. 1). Genesis of these multinucleated structures could be treated as occurrences of endoamitosis,

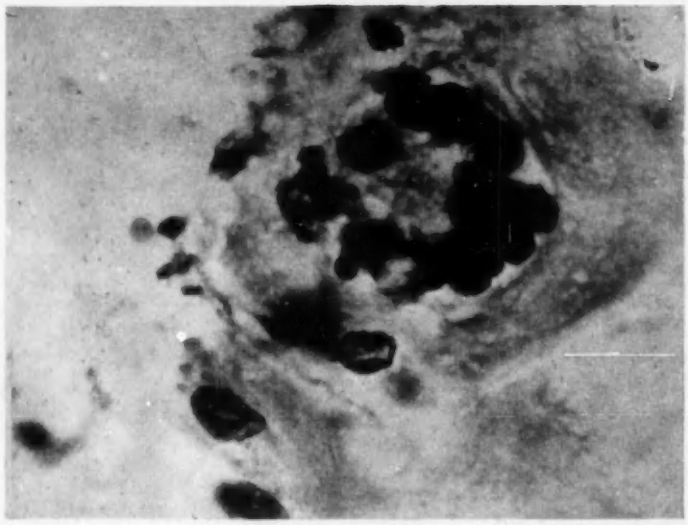

Fig. 1. Genesis of multinucleated structure from a gigantic cytotrophoblastic cell. Inflammation, 1st day. Staining with eosin-hematoxylin. Photomicrograph. Objective, 40 x.

previously described for the placenta [1, 4]. In cytotrophoblastic elements, bordering with the marginal zone, there were frequently observed cases of direct division, but mitosis was absent.

After two days the destructive processes spread through the cytotrophoblast situated beyond the limits of the marginal zone. Cytotrophoblastic elements took stains weakly, their borders were poorly expressed, cytoplasm was vacuolated, the nuclei looked like pale vesicles. The foreign body, the marginal zone, and the adjacent parts of the cytotrophoblast were infiltrated with special leucocytes. Decidual cells partly disintegrated, partly became rounded, and acquired the ability to phagocytosis. In the decidual cells and in the cytotrophoblastic elements amitoses were numerous; mitoses were absent.

On the third and fourth days the leucocytic reaction became weaker. Cytotrophoblastic cells on the border of the necrotic zone expanded strongly and acquired an elongated form. In the form of a narrow layer, they separated the zone of necrosis from the surrounding tissues and they were even wedged in the labyrinthic portion of the placenta (Fig. 2). Together with the above described proliferative occurrences there was observed here the formation of large multinucleated structures. These structures began to appear even during previous stages; they arose from cytotrophoblastic elements via nuclear fragmentation and via the previously described process of division, recalling the picture of endomitosis. In the cytoplasm of cytotrophoblastic cells there could be observed erythrocytic fragments.

Inflammatory reaction of the labyrinthic portion of the placenta. Twelve hr after the introduction of the foreign body, there was formed around it a marginal zone with sharply dilated fetal vascular vessels filled with blood. Leucocytic reaction did not yet begin. Dystrophic changes of the elements in the chorionic symplast of the marginal zone were insignificant and were expressed as a lowering in protoplasmic basophlia and its vacuolization.

At the end of the first day, in the section belonging to the maternal portion, surrounding the foreign body there was formed a zone from nectrotically changed placental tissue, infiltrated with special leucocytes with sharply segmented nuclei. Cytoplasm of the chorionic symplast of this zone was weakly basophilic and contained a fine azurophilic granularity. There were encountered sections where the chorionic symplast was completely necrotized, but the fetal vessels were preserved and were in contact with maternal blood. In the symplast, adjoining the zone of necrosis, the partitions were thinned out, the lacunae were sharply widened and filled with blood. The nuclei of the chorionic symplast of this zone were contracted, the protoplasm was stained weakly and vacuolated.

A somewhat different picture was observed in the region adjacent to the chorionic plate. The elements of this marginal zone were altered to a considerably lesser degree. In the marginal zone there were observed only small necrotic foci, in which were encountered surviving elements of the symplast. From the symplastic net there were isolated individual cells assuming a round or oval shape. Cytoplasm of these cells was sharply basophilic and vacuolated. The dark homogeneous nucleus contained one or two nucleoli. The fetal vessels of the marginal zone were preserved. Leucocytic reaction here was expressed more weakly than in the sections belonging to the maternal palcenta. Symplastic rods, in the sections

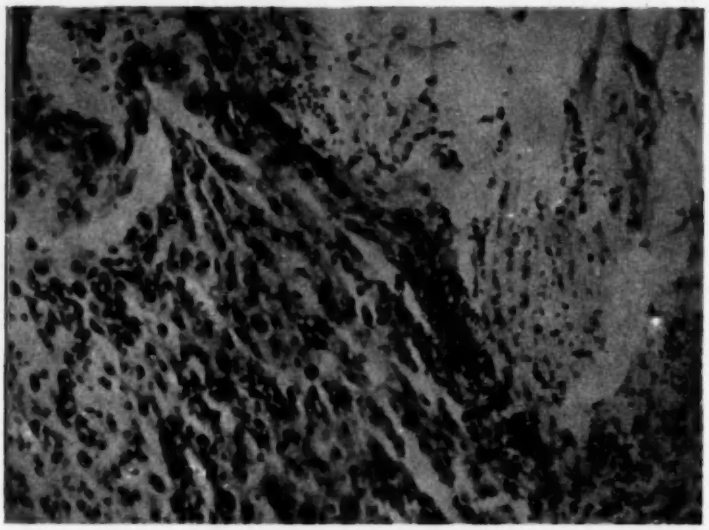

Fig. 2. Expansion of the cytotrophoblastic cells on the border with the foreign body. Inflammation on the third day. Staining with eosin-hematoxylin. Photomicrograph. Objective, 8 x.

bordering with the marginal zone, were thinned out and in places torn. Fetal vessels were dilated and filled with blood; extravasations were present. Symplastic protoplasm of this zone was sharply vacuolated. Sometimes there were observed in it erythrocytic fragments.

In two days the leucocytic reaction became weaker; in the marginal zone the leucocytes formed small aggregates. Placental tissue on the border with the foreign body underwent a quite peculiar change: the chorionic symplast began to expand, its rods became elongated and acquired a fusiform shape, forming around the foreign body a lamellar capsule (Fig. 2). During the process of expansion there was observed a partial separation of the symplastic elements, acquiring cellular structure.

On the third and on the fourth day the foreign body was separated from the surrounding tissues by the continuing expansion of the chorionic symplast. The capsule thickened; trophoblastic elements entering into its composition acquired a more compact distribution. Some of them separated, but the majority remained in a syncytial

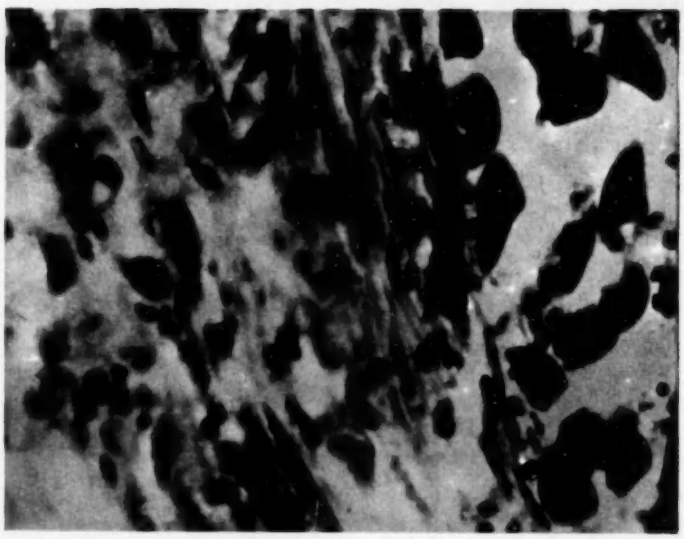

Fig. 3. The lamellar capsule formed around the foreign body by the elements of the chorionic symplast. Inflammation on the fourth day. Staining with azure-2-eosin. Photomicrograph. Objective, 40 x.

union with one another and with the unchanged labyrinth It should be noted that in no inflammatory stage was there observed a noticeable reaction from the endothelium and the adventitial cells of the fetal vessels.

Comparison of the above-described occurrences for the separate parts of the placenta allowed us to conclude that under the conditions of experimental inflammation the most intensely reactive processes were expressed in the extralabyrinthic portion of the placenta and in the adjacent sections. Here, there was an earlier beginning and expansion through larger portions of the cytotrophoblast of the necrotic and dystrophic processes, the leucocytic reaction had a more active course, and first signs of proliferation from the cytotrophoblastic cells appeared more rapidly. In this respect, the study of the injured portion situated on the border of the labyrinthic and extralabyrinthic portions of the placenta is of considerable interest.

In two days there was noted a profuse leucocytic infiltration; from the site of the cytotrophoblast it was expressed particularly sharply. Leucocytes were distributed as large aggregates; on the border with the necrobiotically changed marginal zone they formed an actual leucocytic bank. In the marginal zone, from the side of the labyrinthic portion of the placenta, the leucocytic reaction became considerably weaker; the leucocytic bank was not formed necrobiotic processes proceeded very sluggishly and occupied small sections.

It is in this way that the inflammatory reaction developed in the placenta as the result of introduction into it of a foreign body; in different portions of the placenta it proceeded unequally. The inflammation was expressed most actively in the extralabyrinthic portion of the placenta. A peculiarity of the placental inflammation was the absence of a typical macrophage phase. Separate and rare phagocytizing elements were derived from decidual cells and trophoblastic elements. The inflammatory reaction ended basically on the third day, whereupon at the end of this period there was activation of the proliferation in the trophoblastic elements. As a result of the active overgrowing of the foreign body by cytotrophoblastic cells and symplastic rods there was formed a many-layered capsule separating the injured part from the surrounding tissues. In the encapsulating process of the foreign body, there took place isolation of separate elements of the chorionic symplast which had acquired a cellular structure.

SUMMARY

In experimental inflammation of placenta induced in white rats by passing a silk thread through the placental disk the most active reaction is shown by tissues of the extralabyrinthic portion. In the process of inflammation, there develops leukocytic reaction with almost no macrophagic response. Individual phagocytes are formed mainly from decidual cells. The foreign body is encapsulated by the cytotrophoblastic and chorionic symplast elements.

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ON THE MORPHOLOGY OF EXPERIMENTAL STREPTOCOCCUS INFECTION IN RABBITS WITH A PRIMARY INTRADERMAL FOCUS

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In the literature available to us we did not encounter morphological works dealing with the study of experimental streptococcal infection, and, in particular, of primary foci in the dynamics of their development.

The present work is an attempt to study the local tissue changes arising with the intradermal injection of streptococci. The investigation was conducted microbiologically and on the basis of morphological complexes, and during it we turned our attention to the dynamics of the process. The internal organs were also morphologically studied to elucidate the effect of a local streptoroccal focus on them.

EXPERIMENTAL METHOD

The experiments were carried out on 65 rabbits, 2,000 – 2,900 g in weight. For the inoculation of the animals we used a virulent culture of hemolytic streptococcus group A, Griffith's serological type I (R-phase), obtained from the Division of Microbiology of the Institute of Experimental Medicine, Akad. Med. Nauk SSSR. Eighteen- to twenty-hour cultures of streptococcus, in dishes containing 5% blood agar, were washed off with a small quantity of physiological NaCl solution, carefully mixed by shaking for 1-2 minutes, and diluted with physiological saline in accordance with an optic standard. The culture of hemolytic streptococcus, in the corresponding dilution, was injected into rabbits intradermally in a volume of 0.1 ml. In order to select the optimal infecting dose we set up a series of experiments with different concentrations of the microbial bodies (50,000, 500,000 and 5,000,000). Under the conditions of our experiments a concentration of 5,000,000 cells in the inoculating dose was shown to be optimal. With this dose a clearly manifested focus was formed after 72 hours. Before the animals were inoculated the number of viable organisms in the infecting dose was determined; it usually consisted of 40-50% of the calculated dose. The lateral surfaces of the rabbits were carefully depilated. Two to four foci were created in each rabbit, i.e., 1-2 foci per side, in immediate proximity to the paws. The foci were investigated immediately after inoculation, and then after 2, 4, 8, 24, 36, 48, 60 and 72 hours and 5 days. At the indicated intervals the animals were sacrificed by means of air emboli.

Sectioned portions of the skin containing the foci, stretched out on a cardboard frame, and regional lymph nodes (axillary and inguinal) were fixed in formalin, or, in the last experiments, in Carnu's solution. In 26 rabbits the heart and kidneys were subjected to morphological examination at different intervals of the experiment, and, in certain animals, the periarticular tissue, liver, and spleen as well. The resected portions were imbedded in paraffin and studied after being stained with hematoxylin-eosin, by the method of Van Gieson, and with thionine; in the last series of experiments (12 rabbits, interval after inoculation 36, 48, 60 and 72 hours) we used Schiff's periodic acid reagent (PAS). For the microbiological investigation the foci were sterilely resected in their entirety, cut into tiny pieces with scissors, and ground in physiological saline with a pestle; following this the corresponding dilutions were prepared and seedings were made to dishes containing 5% blood agar for a tally of the number of colonies growing out.

EXPERIMENTAL RESULTS

Macroscopically, after 1 hour (10 foci) and 2 hours (20 foci) following the inoculation, the site of injection of the streptococci differed very little from the surrounding skin; only in certain cases was there observed a small thickening. With microscopic examination one's attention, in both intervals, was called to a disintegration of the collagen fibers and a rounding of the fibroblasts. Scattered leukocytes were noted in three rabbits, in the dermis under the epithelium, after only one hour. After two hours, against the same background of connective tissue loosening and rounding of the fibroblasts, there were, as a rule, scattered leukocytes in considerable numbers, predominantly under the epithelium and around the vessels. We did not succeed in bacterioscopically showing the microbes at these intervals, with the exception of one case, where after only 2 hours focal accumulations

of leukocytes were observed between the bundles of collagen fibers. In this case the microbes were distributed in both the focal accumulations of leukocytes and in the surrounding tissue.

In the site of injection of the streptococci, after 4 and 6 hours (10 rabbits, 20 foci), a minimal thickening of the skin and a hyperemia were inconstantly observed. With histological investigation leukocytes were seen dispersed throughout the entire dermis against the background of connective tissue loosening, predominantly under the epithelium, and along the path of vessels and hair follicles.

The lumens of vessels in the areas of leukocyte accumulation were filled with leukocytes, and their endothelium was swollen. Six hours following the injection of the streptococci a more considerable leukocytic infiltration was observed; the foci were coarser, and the leukocytes were arranged in bands along the route of collagen fiber bundles. Clumps of disintegrating leukocytes were encountered, in which macrophages were admixed. At these intervals the microbes, as a rule, could still not be detected bacterioscopically. The exception consisted of 3 rabbits: in 2 of them, after 4 hours, single, poorly stained, cocci were noted; in only one case was there demonstrated a large number of streptococci, situated in the foci of leukocytic accumulations.

Diffuse thickening and hyperemia of the skin were observed at the sight of injection of the streptococci in the majority of animals 8 hours after the inoculation (10 rabbits, 10 foci). With microscopic examination the same alterations were detected, but, in addition, against this background there were encountered, in many rabbits, rather large foci or leukocyte accumulation between the bundles of collagen fibers.

Twenty-four hours after injection of the streptococci, in all the rabbits (10 animals, 10 foci) except one, dense, slightly elevated infiltrates were noted at the site of injection. Upon microscopic investigation, large foci of leukocytic infiltration were discovered. As in the foregoing experimental interval, the leukocytes were arranged as dense masses between the bundles of collagen fibers, but areas were encountered where they lay in a continuous mass, in which there were already undifferentiated connective tissue fibers. The majority of leukocytes were in a state of disintegration; in two cases streptococci were discovered, both lying free and phagocytized.

Thirty-six hours (20 rabbits, 20 foci) and 48 hours (20 rabbits, 20 foci) after the inoculation, circumscript foci of thickening were formed at the site of injection of the streptococci in the majority of animals, 0.2 to 1 cm in diameter. Upon histological investigation, circumscript foci of leukocutic infiltration were present in almost all the animals, in the form of one single, or several small, foci, presenting themselves as generating abscesses. In several places the border of the abscesses wss very clear, but lacking marked proliferative alterations on the side of the surrounding tissue. In 6 cases streptococci were detected in the leukocytic accumulations.

At the interval of 60 hours following the injection of streptococci (20 rabbits, 20 foci), in almost all cases there was noted, at the site of injection, slightly raised, sometimes pale, sometimes hyperemic foci of thickening up to 1 cm in diameter. Upon microscopic investigation the foci of leukocyte accumulation were shown to be larger than in the previous intervals; in many cases they reached the muscular layer of the skin. The changes in the tissue surrounding the abscesses were the same as in the previous intervals of the experiment. The bundles of collagen fibers in the foci were either preserved in the form of isolated fragments or were completely absent, and the entire basic mass of the focus consisted of disintegrating leukocytes and macrophages.

Seventy-two hours after the inoculation (28 animals, 28 foci), the foci at the site of injection of the streptococci were larger, reaching up to 1.5 cm, many of them with an ulceration at the surface. Upon histological investigation, formed abscesses were discovered in half the cases. In isolated cases a dense macrophagal-leukocytic infiltrate extended to the muscular layer of the skin. The deep veins of the skin were thrombosed. The epithelium over the abscesses became necrotic. Streptococci were discovered in the disintegrating mass of the exudate in 2 cases. In the remaining cases the foci were approximately the same as the form they had taken in the preceding intervals. Proliferative changes were absent or were extremely weakly manifested. The development of granulation tissue about the periphery of the abscesses was observed, in isolated cases, only after 120 hours following the inoculation (10 rabbits, 20 foci).

Testing the skin with the periodic acid- Schiff reaction, we did not detect any expressed changes in the connective tissue at the different experimental intervals. In the regional lymph nodes of the cutaneous foci of injury the changes were inconstant, irregular, and weakly manifested. Enlargement and hyperemia of the lymph nodes was observed in many of the rabbits, but these alterations did not depend on the interval of the experiment. Upon microscopic investigation, the lymph nodes, during the early intervals, did not differ from the nodes in the control experiments (4 rabbits). Indeed, only after intervals of from 48 to 120 hours were changes discovered in the lymph nodes in 18 of the animals, generally corresponding to the extent of the changes in the skin. Forty-eight hours after the injection of the streptococci there was noted a dilatation of the marginal sinuses, an accumulation of light, large cells and isolated leukocytes in them, and occasional disintegrating cells. After 60 and 72 hours clearly expressed dissociation of the cells was observed in the sinuses. In several cased disintegration of the lymphocytes was noted in the follicles. After 120 hours we observed a decrease in the size of the follicles, a dilatation of the sinuses, and filling of the latter with large cells. In the marginal sinuses, macrophages were encountered with an

Number of Microbes in the Foci According to the Intervals of the Investigation

No of the experiment	Intervals of the Investigation (in hours)							
	immediately following inoculation	2	4	8	24	36	48	60	72
1	3.2×10^3	44.8×10^3	165×10^3	---	---	---	---	---	---
2	---	108×10^3	240×10^3	430×10^3	1.5×10^6	---	---	---	---
3	---	---	---	---	---	2.1×10^6	4.7×10^6	6×10^6	4.3×10^6

adiposity of the protoplasm and with phagocytized products of nuclear decomposition. Microbes were not detected in the lymph nodes bacterioscopically.

The data of the series of experiments is presented in the table, illustrating the dynamics of an intradermal streptococcal focus in rabbits as studied by microbiological investigation.

Thus, we see that immediately after the inoculation the number of viable microbes in the focus diminishes considerably. Beginning from 2 hours an intensive multipliation of the microbes is observed, attaining its maximum 60-72 hours after the injection of the stroptococci.

The results obtained permit characterizing the focus of infection arising, under our experimental conditions, in the skin of the rabbit by the dynamics of its development.

It merits attention that up to 72 hours inclusively, according to both the morphological and microbiological data, the local process progresses. It is interesting that under the applied experimental conditions proliferative changes on the part of the tissue surrounding the abscess were either absent or extremely weakly manifested in the early intervals (up to 72 hours), and only after 120 hours following the injection of streptococci, when the microbes were not dispersing and, morphologically, the process had abated, was a proliferative reaction observed. Upon morphological examination of the internal organs, dystrophic changes were discovered in the heart, kidneys, and liver. The absence of an increase in these changes with a prolongation of the intervals in the experiment speaks for the generation of the dystrophic processes not being related to an effect of the streptococcal infection. In several rabbits hyperplasia of the follicles or the pulp of the spleen was observed, again without relation to the experimental intervals. Changes in the spleen in the form of a disintegration of the lymphocytes, characteristic for this infection, were not noted. Under the given experimental conditions no other changes were observed in the internal organs as a result of the streptococcal infection, especially according to the treatment of the sections with the periodic acid- Schiff reagent. This testifies to the mild course of this infection, which is also corroborated by the changes developing in the intradermal foci.

SUMMARY

The development of experimental intracutaneous streptococcus foci (178 in number) in 65 rabbits was subjected to a complex (morphological and bacteriological) investigation; the effect of these foci upon the condition of internal organs was also studied. Group A (first serological type) streptococcus culture was administered according to Griffiths. The experiments lasted from 1 to 120 hours. Local infectious processes progress up to the time when the abscesses are formed – 72 hours. A significant proliferative reaction around these foci is noted only after 120 hours, when it is already impossible to isolate the microbes from the foci. There were no changes in the internal organs connected with the presence of the streptococcus foci.

AN ELECTRIC THERMOESTHESIOMETER

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Studies of the receptor functions of the skin are of greate importance for medical research workers in various specialties. Although it is comparatively simple to determine tactile and pain thresholds, we have encountered considerable technical difficulties in determining temperature thresholds. At first we used a water thermoesthesiometer, but experience has shown that a good deal of time is required to determine warmth and cold thresholds, and that the conditions for using this instrument are cumbersome. What is more, the determination is not sufficiently accurate. But after trying a number of different models, we have been successful in building an electric thermoesthesiometer that is very simple to construct and use, and is also sufficiently accurate. It can be used both in the laboratory and also under any other conditions, with no restrictions and no additional instruments. With this device, we have examined about 350 persons, in the normal state and in various disease conditions. The device is suitable for extensive use in practical medical work.

The electric thermoesthesiometer (see figure) is in the shape of a pistol. The barrel contains a mercury thermometer with a range extending to 100° C. Movements of the mercury may be viewed through an opening in the barrel. The mercury reservoir protrudes from the barrel, and is encircled for three quarters of its length by a spiral of Nichrome wire. The tip of the mercury reservoir, which makes up one-fourth of its length, is exposed and serves as a thermal stimulator. Wires run through the barrel from the spiral to the handle, which contains a pocket-flashlight battery with a voltage of 3.7 v. When the circuit is closed the nichrome spiral heats up, warming the mercury reservoir. The temperature at the end of the reservoir is read from the scale of the thermometer, at the same time that it is giving rise to subjective sensations on the part of the individual being examined. To determine the cold threshold, we cool the end of the mercury reservoir with ethyl chloride, and it is then possible to obtain any desired low temperature at the end of the mercury reservoir by warming it. The flashlight battery is changed in accordance with the amount of use the instrument receives.

SUMMARY

The author offers an electric thermoesthesiometer of his own design. The device may be used in all conditions for the determination of thermal and cold thresholds. A

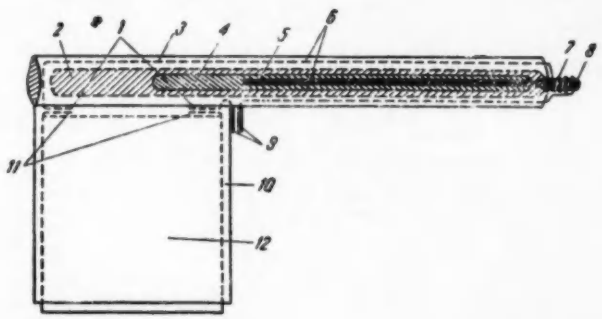

Fig. Electric thermoesthesiometer. 1) Contacts for battery; 2) mercury thermometer; 3) barrel of thermoesthesiometer, for thermometer and wires; 4) opening for determination of thermometer readings; 5) mercury column; 6) wires from spiral; 7) Nichrome spiral around mercury reservoir; 8) end of mercury reservoir, making contact with skin; 9) contacts for closing circuit; 10) combination handle and battery case; 11) battery terminals; 12) pocket flashlight battery, 3.7 v.

mercury thermometer graduated to 100°C is inserted into the barrel of the instrument. The movement of the mercury column may be seen through the slots in the barrel.

The mercury reservoir is surrounded by coils of nichrome wire. Its free end acts as a thermostimulus. The coil is heated by current from a pocket flashlight battery fitted into the handle of the instrument. When in contact with the instrument the heating of the mercury reservoir provokes a subjective feeling in the patient under observation. In determining the cold threshold, cooling of the end of the mercury reservoir by ethyl chloride is combined with its heating. The temperature of the thermostimulus is read off on the thermometer scale.

ON THE STUDY OF MOTOR DEFENSE CONDITIONED REFLEXES

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The method of motor defense conditioned reflexes is widely used in modern physiology laboratories. It was first employed by V. P. Protopopov [10], and was later radically improved by other authors. As shown by subsequent work (mainly that of V. P. Petropavlovskii [8] and L. S. Gambaryan [4]), the main essential defect of Protopopov's method was that despite all the animal's response activity, it could not escape the painful stimulus: the flow of current through the paw. The result was that chaotic movements and lifting of the paw between signals developed, which often led to neurotic conditions. Taking this fact into account, V. P. Petropavlovskii introduced a substantial change in the method. In his modification, the current was automatically turned off as soon as the dog lifted its paw to a definite preassigned height, and was automatically turned on when the paw was below this level.

In using Petropavlovskii's method we came to a point where it was necessary to compare conditioned reflex activity in different dogs and in each dog individually during different periods of investigation. Which motor responses should be regarded as greater and better responses and which as smaller and worse? The question arose as to the proper criteria for evaluating and comparing motor conditioned refelexes.

Motor responses studied by Protopopov's method can in general only be evaluated from the quantitative standpoint (over-all description of motor activity), since the animal's response does not free it from the painful stimulus – i.e., does not perform its adaptive role. In Petropavlovskii's method, besides the quantitative characteristics of the reflex, it is also possible to evaluate its biological quality (its adequateness), which in itself is of fundamental importance in evaluating higher nervous activity, as has been repeatedly emphasized in the writings of E. Sh. Airapet'yants [1, 2], P. K. Anokhin [3], and others.

But in published reports to date there is no single system of evaluation, no single scale which would combine the quantitative and qualitative indices of the motor conditioned reflex and at the same time reflect gradations in these indices. Thus, comparison of different motor reflexes and complete evaluation of these reflexes have been impeded.

With this as a starting point, we have employed a scale from zero to seven in our work, for recording both quantitative and qualitative indices of the motor defense conditioned reflex. Our chief criterion in evaluating this reflex was the history of its development in the animal. Here we took into consideration I. P. Pavlov's statement that as the defense reaction develops it changes in a definite sequence: from its lowest primitive form to the most "expedient" form [6]. In fact, despite all the differences which we have observed in different animals in the process of developing conditioned reflexes by Petropavlovskii's method (disappearance or, on the other hand, prolongation of some phases, etc.), there is a similarity in the main sequence of stages: At first, as we all know, frequent phasic jerks are observed, which assume an increasingly well defined tonic form from one trial to the next. The chaotic, "inexpedient" reaction gradually becomes an "expedient" (under the given conditions) tonic conditioned reflex.

A special analysis of this complex motor reaction [11] has shown that when lifting is phasic, an ordinary conditioned reflex develops in response to the conditioned signal, and when lifting is tonic, a conditioned-conditioned reflex develops. For this reason, phasic lifting, even with a large amplitude of movement, must evidently be rated lower as being less complex and less complete than sharply defined tonic lifting, even though the latter is of small amplitude. In addition, the adequateness of the reflex – i.e., its ability to protect the organism from "threatening danger" (under these conditions – raising the paw to the level at which the current is cut off) – as a qualitative index of conditioned reflex activity is a fundamental and deciding factor in evaluating the reflex, and we have attempted to reflect this in the following scale.

Seven-point scale (characteristics of motor defense conditioned reflex)

Rank	Height and character of motor reaction
0	No lifting
1	Shifting from one foot to the other and back
2	Low, phasic (i.e., not sustained) lifting
3	High phasic lifting
4	Low, tonic (i.e., sustained) lifting
5	Inadequate tonic lifting
6	Adequate, well-defined tonic lifting
7	Above adequate level, well-defined tonic lifting

Each rank corresponds approximately to a definite stage in the development of the conditioned reflex; a higher rank corresponds to a more complete, more adequate conditioned reflex. We are aware that this scale is still far from the desired degree of perfection. But as an attempt to give a simultaneous account and comparison of the quantitative and qualitative aspects of the conditioned motor reaction, it has been used with success in our work.

In recent years, valuable improvements have been made in the method of conditioned motor defense reflexes [4, 9, 12, etc.]. All the authors in question have devoted most of their attention either to the system for giving the unconditioned stimulus, or to the response activity of the animal, or to the recording system, whereas the system for signalling – i.e., for giving the conditioned stimulus – has remained almost unchanged since the time of Protopopov's first work.

In the alimentary, acid, and other methods the conditioned stimulus is necessarily turned off together with the unconditioned stimulus; if not, as is well known, it begins to lose its significance as a signal, and extinctive inhibition develops. In Petropavlovskii's method, the conditioned stimulus continues to act after the current stops flowing to the paw.* Thus, the conditioned stimulus over a period of several seconds is first a signal of the current (while the paw is down), and then a "signal of the absence of current" – i.e., a signal that intentionally should not be reinforced with the electric current so long as the paw is lifted above the adequate level.

Meanwhile, the conditioned stimulus for the defense reaction is not so harmless an agent for the animal that it may easily and quickly change its biological significance. Thus, as Pavlov noted, "the conditioned stimulus is a pure substitute, a pure surrogate for the unconditioned stimulus. In a conditioned elementary reflex, the animal may lick a flashing light bulb, may, so to speak, catch a sound itself in its mouth and eat it, licking its lips and making chewing sounds with its teeth as though it were dealing with food itself" [7]. In our own experiments with defense reflexes, we have also observed this.

For this reason, the most adequate defense reaction for the animal will be that reaction which delivers it both from the action of the disturbing factor itself and from the signal of the disturbing factor. (In this respect, the general idea introduced by Petropavlovskii in improving Protopopov's method, though correct as far as it went, proved logically incomplete, and its embodiment in the method was also incomplete.)

Guided by this basic proposition, we employed a system in which, when the paw was lifted to the adequate level, the dog turned off not only the unconditioned stimulus but also the conditioned stimulus Under these circumstances the animal not only protects itself from the disturbing agent, but also removes the signal for this disturbance. When the signal for the current is turned off, a supplementary conditioned stimulus – an illuminated translucent shield – is automatically turned on; it continues to signal as long as the paw remains "under the current" i.e., as long as it is possible for the dog to receive a shock if it lowers its "working limb." If, during this period, the paw is lowered beyond the adequate level, the unconditioned stimulus and the ordinary conditioned stimulus are turned on at the same time that the translucent shield is turned off. The total period of signalling – i.e., the total time of application of the ordinary and supplementary conditioned stimuli – lasts twenty seconds; the current is turned on five seconds after the ordinary conditioned stimulus is turned on. As is shown by the experiments described below, this type of system helps the animal develop conditioned reflexes, and creates more natural experimental conditions.

EXPERIMENTAL METHODS AND RESULTS

We performed the first series of experiments on six dogs in an acoustically deadened room, by Petropavlovskii's method. The voltage applied to the paw from the induction coil was about 30 v, and the current was approximately 15 ma. The motor and respiratory components of the response were recorded with a kymograph. The following conditioned stimuli were used: a bell, a buzzer (differential stimulus), a Pavlov-type tactile stimulator, and a light. Although a large number of trials were made (considerably more than a hundred), we observed more or less clearly defined conditioned reflexes (corresponding to the sixth rank on our scale) in only one dog – and even with this dog we often observed very strong jerking movements in response to the conditioned stimulus, and in the intervals between signals we noted prolonged, large-amplitude lifting of the paw. In the other five dogs, the conditioned reflexes were chaotic and unstable and sometimes disappeared; in the intervals there was barking, and the dogs were

* Strictly speaking, the conditioned signal is still reinforced even in this position (by kinesthetic stimulation from the lifted paw [5, 11, etc.]). But in this case reinforcement is qualitatively different form reinforcement by electric current, about which we shall have more to say.

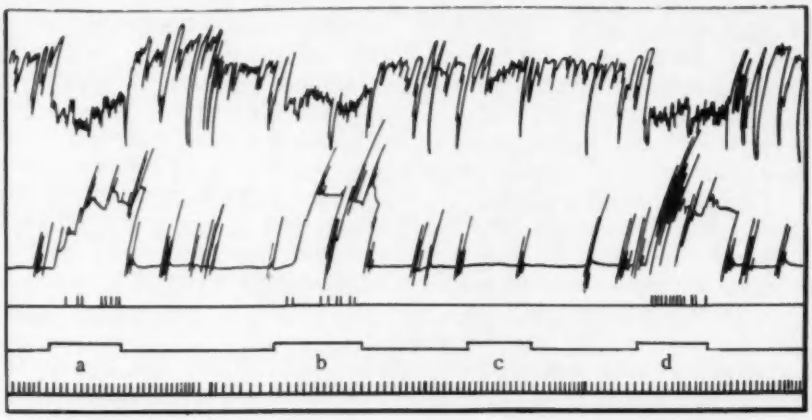

Fig. 1. Motor defense conditioned reflexes, developed by V. P. Petropavlovskii's method. Dog: Ryzhii. Experiment 97 (March 28, 1958). The starting conditioned stimulus – the signal of the current – is applied throughout the entire run. Curves (top to bottom): respiratory movements; lifting of paw; unconditioned stimulus; conditioned stimulus; time (2 seconds). a) Bell (+); b) Tactile stimulator (+); c) Buzzer (-); d) Light (+).

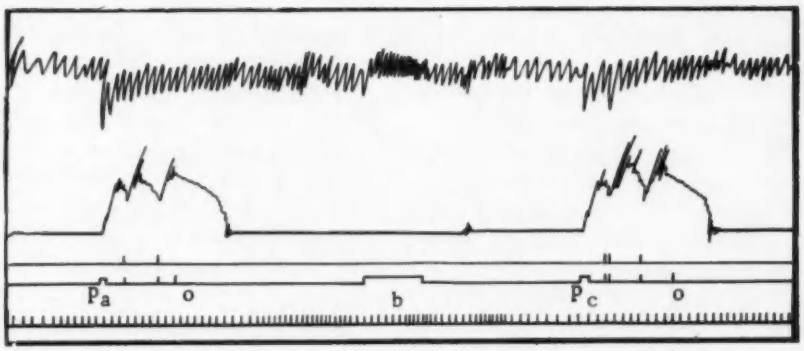

Fig. 2. Motor defense conditioned reflexes, developed by our new modification of the method. Dog: Dik. Experiment 23 (January 3, 1959). The starting conditioned stimulus – the signal of the current – is turned off by the animal itself. There is no special marker for the action of the stop-signal (illuminated translucent shield) on the kymograph record; it turns on at the instant the starting conditioned stimulus is turned off, and vice versa. Significance of records same as in Fig. 1. p-period of delay of conditioned reflex (adequate level); o-instant when experimenter turned off all stimuli, terminating run. a) Bell (+); b) Buzzer (-); c) Light (+).

constantly excited throughout the entire experiment (Fig. 1).

We performed a second series of experiments on three dogs, using our new variation of the method. The over-all experimental conditions remained the same, but the conditioned reflexes, as a rule, were marked by a high degree of definition and adequateness, and the animals behaved much more calmly during experiments (Fig. 2).

Thus, our Pavlovian guiding principle concerning the biological significance of conditioned reflex activity, together with an experimental comparison of the two versions of Petropavlovskii's method, has led us to prefer the version that we are proposing. Besides the advantages indicated, with our modification it is possible to determine the delay period of the adequate conditioned reflex – i.e., the time from the moment when the conditioned stimulus (the signal of the current) is turned on to the moment when the paw is raised to the adequate level – with quite a high degree of accuracy (to 0.1-0.2 seconds). Since this lifting of the paw coincides precisely with the turning off of the conditioned stimulus (which is also

marked on the kymograph record), the delay period is readily measured with calipers (see Fig. 2, distance p). In this way the technical difficulty of precisely determining the delay period, which Petropavlovskii himself noted with regret, is greatly reduced.

In motor defense conditioned reflexes, one interesting and important feature should be emphasized, which pertains to the specific role of the conditioned stimulus after the paw is lifted to the adequate level. In an actual display of a previously developed conditioned reflex, two qualitatively different periods can be observed: 1) When the conditioned stimulus is turned on, it signals the approach of the current – it evokes a conditioned lifting of the paw; 2) the paw is lifted above the adequate level – now the conditioned stimulus is no longer the signal of the current, since the current was turned off earlier by the animal itself. Therefore, during the second period, the biological role of the conditioned signal is qualitatively different from what it was during the first period.

The same can be said of the organism's response activity and over-all condition. This is evidenced not only by the behavior of the animal (for example, a momentary quieting of the excited animal after the paw is lifted), but also by the autonomic component of the conditioned reflex (see the record of respiratory movements in Fig. 2). The defense conditioned stimulus evokes a sharp, deep inspiration, which has been observed repeatedly by many authors. When the paw is lifted, respiration gradually returns to normal. † Whereas, during the first period, the conditioned stimulus sets the defense apparatus in motion, as it were (in particular, local movement of the stimulated limb ensues), in the second period, on the other hand, the conditioned signal arrests this movement at the height of lifting – the animal freezes, so to speak, in an unnatural pose. Therefore, in contast to ordinary starting signals, the conditioned stimulus that is applied after the paw is lifted plays a correcting role. It might be called a stop-signal.

Thus, in our experiment, states of the animal that are qualitatively different biologically are signalled by different stimuli. Besides what we have said above, this allows us to study the characteritstics of motor defense conditioned reflexes more fully.

SUMMARY

A modification of Petropavlovskii's method of studying motor defense conditioned reflexes is suggested. A seven-point scale is employed for better evaluation of conditioned-reflex activity, taking into consideration the relationship between the quantitative and qualitative indices of the conditioned motor reaction, and for comparison in graphic form of the results of experiments by the same motor method. To create more natural experimental conditions for the animal, the author suggests a system in which the animal, by raising its paw, turns off both the unconditioned and conditioned stimuli, and simultaneously turns on an illuminated translucent shield, which plays the role of a correcting signal.

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† It is noteworthy that when the ordinary conditioned stimulus continues to be applied after the paw is raised, respiration shows either a limited tendency or none whatever to return to normal (see Fig. 1). But if the signal of the current is turned off when the paw is raised, respiration quickly returns to normal (see Fig. 2).

A METHOD OF OBTAINING BLOOD FROM WHITE MICE

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White mice are widely used for biological research. The possibility of obtaining large numbers of animals, their cheapness, and the ease of rearing and care all go to make mice irreplaceable experimental animals. White mice are no less widely used in the manufacture of bacterial preparations as controls of nontoxicity of vaccines and sera, the antigenicity of vaccines, and so on. It has recently been shown that the testing of the antigenic properties of tetanus [3, 4] and gas gangrene [1] toxoids may be successfully carried out also in white mice.

One of the disadvantages of work with white mice is the impossibility of obtaining blood for determination of the antibody level. For this reason tests of the antigenicity of vaccines and toxoids are based either on computation of the LD_{50} [5] or the effective index [2], or on determination of the rate of survival after inoculation of the animals with definite doses of toxins [1, 3, 4]. By puncture of the heart, amputation of part of the tail, or puncture of the paws or ear it is possible to obtain 2-3 drops of blood, which is insufficient for titration, and only by decapitation of mice may 0.3-0.5 ml of blood be obtained. In cases in which it is necessary to observe the course of antibody production, all investigations must therefore be carried out on other animals – guinea pigs and rabbits – and not on mice.

We considered it to be of practical importance to develop a method, suitable for general use, of obtaining blood from white mice in a sufficient quantity for serological and immunological investigations. For this purpose we adopted the method of obtaining blood from rats described by Woodruff and coworkers [6], based on the use of a lowered pressure.

EXPERIMENTAL METHOD

The glass receiver which we used (Fig. 1 and 2) has three openings. Into opening A, firmly closed with a cork, is inserted the mouse's tail; the opening B is connected to a manometer and motor for aspiration of air, and to opening C is attached the tube for collecting the blood.

The technique of obtaining blood is simple: The mouse's tail is smeared with xylene in order to dilate the caudal veins, the tip of the tail is cut off with sharp

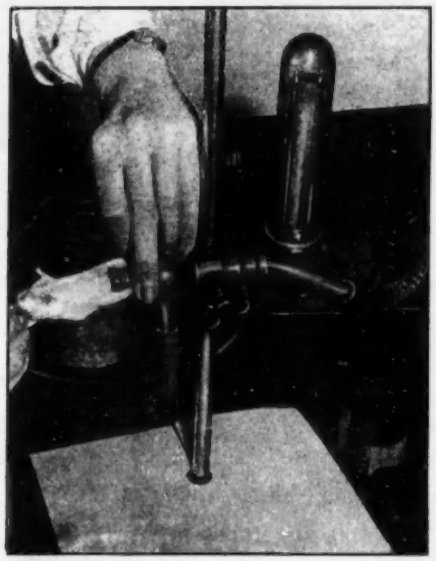

Fig. 1. Glass receiver for taking blood from white mice (general view).

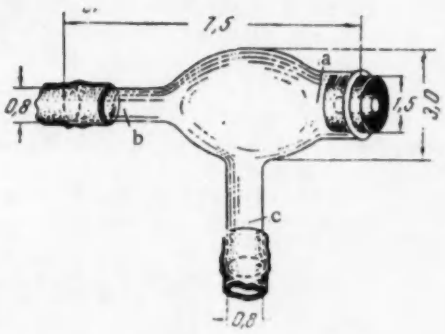

Fig. 2. Diagram of the glass receiver for taking blood from white mice. A) Opening into which the mouse's tail is placed; B) opening to the motor for aspiration of air; C) opening to the tube for collecting the blood (the internal dimensions are given in cm).

scissors, and to it is applied a drop of 5% sodium citrate solution. The mouse is then fixed firmly to the apparatus so that its tail falls into the opening C. The motor is set in motion and the collection of blood begins.

The first series of experiments was carried out on white mice in order to clarify the details necessary for the satisfactory obtaining of blood. It was discovered that the posterior part of the mouse's body must be firmly pressed against the opening A so that a pressure of 20-40 mm Hg was created in the receiver. At a higher (over 40 mm Hg) or lower (below 20 mm Hg) pressure, blood is obtained very slowly or not at all. If the opening A is closed with a rubber stopper or its size is reduced so that it fits closely to the posterior part of the mouse's body, a very low pressure (2-5 mm Hg) is created in the receiver, the vessels are compressed and blood does not flow. It was therefore found most suitable to use a cork stopper, which does not compress the vessels of the posterior part of the mouse's body and permits the entry of air into the receiver so that the pressure inside varies between 20 and 40 mm Hg. The mouse's tail must either be on the wall of the receiver or fall towards the opening C; should it enter the constricted part of the receiver, towards the opening B, blood will also cease to flow. In order to obtain a translucent, nonhemolyzed serum, it is essential that the walls of the receiver and tube be smooth; this is achieved by coating them with paraffin wax and by the use of short tubes (up to 5-9 cm). Under these conditions a perfectly translucent serum was obtained over a compact blood clot (Fig. 3).

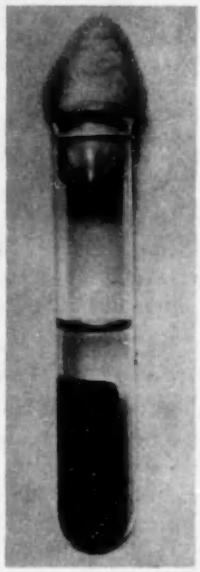

Fig. 3. Tube containing blood taken from white mice.

EXPERIMENTAL RESULTS

The results of taking blood from 100 mice by the method described are given in Table 1 and compared with those obtained by decapitation.

It may be seen from the data (Table 1) that, by the method described, it is possible to obtain from mice 0.2-0.5 ml of blood, with an average of 0.3 ml from each animal, or the same volume as by decapitation. The time required for taking blood is from 2 to 5 minutes per animal. It must be emphasized that the taking of blood by this method does not affect the condition of the mice—they remain active and healthy; not once have dyspnea, fits or death of the animals been observed. This is a considerable advantage of the method described, for the mice may be used for subsequent investigations.

In the next experiment, carried out on 10 mice which were immunized with 1 ml of tetanus toxoid, we investigated the possibility of taking blood repeatedly (every 5 days). As a check on the condition of the animals, they were systematically weighed and the blood examined. In order to compensate for the loss of blood taken, an injection of twice the volume of physiological saline was given to the animals soon after bleeding. The results obtained are given in Table 2.

The results given in Table 2 show that it is possible for blood to be taken repeatedly from white mice.

On each occasion from 3.1 to 3.8 ml of blood could be taken from 10 mice. In the course of five takings of blood from each mouse, 1.5-2.0 ml of blood was obtained; the animals' condition nevertheless remained satisfactory and they did not lose weight. It is true that these repeated

TABLE 1. Results of Taking Blood from Mice by Various Methods

No. of mice	Site of taking blood	Volume of blood from 1 mouse (in ml)	Time (in min)	Pressure (in mm Hg)
100	Caudal vessels	0.2–0.5 (average 0.3)	2–5 (average 2½)	20–40
10	Cervical vessels by decapitation	0.3–0.5 (average 0.4)	2–3 (average 2½)	–

TABLE 2. Results of the Investigation of Repeated Taking of Blood (ever 5 Days) from Mice

Index		Taking of blood every 5 days				
		1st	2nd	3rd	4th	5th
Volume of blood from 10 mice (in ml)		3.1	3.6	3.2	3.8	3.5
Time required for taking blood (in min)		35	30	28	23	22
Length of tail cut off (in mm)		8-12	3-4	3-4	3-4	3-4
Condition of mice		Satisfactory				
Average weight of each mouse (in g)		16.5	16.5	16.5	16.5	16.5
Mean Hemoglobin level (in %)		96	67	69	–	37
Mean red cell count (per mm^3)		6,520,000	6,420,000	5,960,000	–	3,920,000
Mean leukocyte count (per mm^3)		9,950	9,750	9,650	–	9,450
Leukocyte formula (mean)	Lymphocytes	64	66	65	–	59
	Neutrophils	27	27	26	–	35
	Monocytes	8	7	9	–	6
	Eosinophils	1	–	–	–	–
	Basophils	–	–	–	–	–

TABLE 3. Effect of Repeated (everv 48 Hours) Taking of Blood on the Condition of Mice

Index		Taking blood every 48 hr				
		1st	2nd	3rd	4th	5th
Volume of blood from 10 mice (in ml)		3,4	3,1	3,2	3,9	3,3
Time required for taking blood (in min)		30	33	41	27	21
Length of tail cut off (in mm)		8–12	3–4	3–4	3–4	3–4
Condition of mice		Satisfactory				Dyspnea, quickening of the heart rate; 2 mice died
Average weight of each mouse (in g)		17,6	16,9	16,2	14,6	13,5
Mean hemoglobin level (in %)		60	—	37	—	32
Mean red cell count (per mm^3)		5 245 000	—	3 580 000	—	3 740 000
Mean leukocyte count (per mm^3)		7 300	—	7 560	—	8 630
Leukocyte formula (mean)	Lymphocytes	64	—	65	—	64
	Neutrophils	33	—	29	—	32
	Monocytes	3	—	4	—	4
	Eosinophils	—	—	2	—	—
	Basophils	—	—	—	—	—

takings of blood led to the development of anemia: The hemoglobin fell to 37%, the red cell count to 3,000,000, and the leukocyte count and formula remained practically unchanged.

A similar experiment was carried out on ten mice, immunized with 0.5 ml of perfringens toxoid, in order to see if it was possible to take blood more frequently (every 48 hours). The results obtained are shown in Table 3.

As the results in Table 3 show, more frequent taking of blood is possible. In these circumstances a volume of blood sufficient for titration–from 3.1 to 3.9 ml – may be obtained each time from 10 mice. Despite such frequent taking of blood, the animals' condition was satisfactory; only after the fifth taking of blood did the mice show dyspnea and a quickening of the heart rate; 2 mice died. It must be pointed out that such frequent taking of blood leads to a considerable loss of weight and to the rapid development of a severe anemia.

SUMMARY

The authors developed a method of obtaining blood (nonhemolyzed serum) from the caudal blood vessels of white mice in amounts sufficient for serological and immunological examinations. A glass bulb was connected to a suction motor to reduce the pressure. This method permits systematic and repeated blood drawing (3.1–3.9 ml of blood from 10 mice)–5 times at intervals of 2-5 days. Blood drawing performed by method does not affect

the condition of the mice and permits them to be used for other investigations.

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A BIOLOGICAL METHOD OF DETERMINATION OF PREPARATION 1314 Th (THIANIDE) IN THE BLOOD

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Among the antituberculous chemotherapeutic drugs synthesized in recent years, the preparation 1314 Th, the thioamide of α-ethylisonicotinic acid, which was produced in France, is of particular interest [3, 4]. An original method of synthesis of this drug, which we call thianide, has been developed in the Institute of Pharmacology and Chemotherapy of the AMN SSSR. Thianide was obtained in the form of a water-insoluble base and of a readily soluble hydrochloride. The activity of these two preparations is practically equal [1].

Thianide possesses a very narrow spectrum of activity mainly against the various species of mycobacteria of tuberculosis [1]. In the study of certain aspects of the mechanism of action of thianide, its distribution in the body and so on, the tubercle bacillus is very unsuitable for use as a test organism, and often impossible on account of its pathogenicity or, in particular, its slow growth

We sought a suitable test organism for these purposes among the group of acid-fast saprophytes. We found here a strain (M. chelonei Friedman) sensitive to thianide and growing on Sauton's and Hiss's media and in Hottinger's broth in the course of 2-3 days. We attempted to develop a method of determination of thianide in the blood using this microorganism.

The strain was grown on Helberg's medium at 37° for 4-5 days. To obtain material for seeding, a culture was emulsified in physiological saline with glass beads, and diluted to 500,000,000 bacterial cells per ml according to the GKI bacterial standard.

In order to select the most suitable medium for titration of thianide, we determined the minimal suppressing concentration (MSC) of its hydrochloride on Sauton's and Hiss's media and on Hottinger's broth at pH 7.3. Friedman's bacillus was more sensitive to thianide on Hiss's medium and on Hottinger's broth (MSC 7.8-13.6 γ/ml), and the second of these was chosen, for the film formed on it was well defined after only 2 days. Trials with Hottinger's broth at pH 3.0, 4.0, 5.0, 6.0 and 7.3 showed that the best results were observed on broth at pH 5.0, and this was used in the later work.

The study of the effect of the dose of microorganisms on the results of titration showed that the most suitable dose was one of 1,000,000 bacterial cells per ml of medium. In this case, when the results were read on the second day, the MSC had a value of 7.8 γ/ml, and in other cases of 3.9 γ/ml.

In accordance with the aims of our work, we studied the effect of serum on the activity of thianide. Experiments were carried out with ox, horse, and rabbit sera, in two variants: the drug was dissolved in serum and then titrated in Hottinger's broth, or it was dissolved in physiological saline and titrated in Hottinger's broth to which 20% of serum had previously been added. In both cases the activity of the drug was lowered three- or fourfold. No significant difference was found in the degree of reduction of the activity of thianide in the two variants of the experiment or with the different sera.

In view of these findings, we decided to use trichloroacetic acid (TCA) for the determination of the thianide in the blood [2]. Experiments were carried out in which 2.7 ml of citrated rabbit's blood was mixed with 0.3 ml of thianide, dissolved in physiological saline. After exposure at 37° for 1 hour, to 3 ml of the mixture was added 1.5 ml of 20% TCA. The mixture was then shaken and centrifuged, and the activity of the supernatant fluid, neutralized with a 10% NaOH solution, was determined. As a control we used thianide in the same concentration as in the experiment, but dissolved in physiological saline. The results of the determinations in the experimental and control series were mainly in agreement, or differed by only one dilution.

In view of these findings, we determined the total thianide concentration in the blood of mice in accordance with the following scheme. Blood from the animals was collected in graduated centrifuge tubes containing sodium citrate in a dose of 16 mg per 1 ml of blood. After mixing the contents, we added 20% TCA to these tubes – 0.5 ml to 1 ml of blood. The mixture was centrifuged for 20-30 minutes at 1500 rpm. After neutralization, the supernatant fluid was titrated in Hottinger's broth at pH=5.

To make the determination more accurate, we used serial dilutions in which the diluting factor was 1.5 instead of 2, and this gave clearer results. As a standard we used a solution of 1 mg thianide hydrochloride in 1 ml of physiological saline, and treated in the same manner as the blood.

As an illustration we give the following example. After injection of thianide hydrochloride intravenously into mice (weighing 20-22 g) in a dose of 1 mg per animal, 5 minutes after injection the blood concentration of thianide was 39 γ/ml, and 30 minutes after injection, 25 γ/ml.

The method described is not specific for blood alone, but may be used in principle for determination of the thianide content in other body fluids and tissues.

SUMMARY

The acid-resistant saprophyte Mycobacterium chelonei Friedman is sensitive to 1314 Th (Thianide)—an antituberculosis preparation. The authors developed a method of determining thianide in the blood with the aid of this microorganism.

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*Original Russian pagination. See C. B. translation.

A METHOD OF PRODUCTION OF CIRRHOSIS OF THE LIVER WITH SPLENOMEGALY AND ASCITES IN RABBITS BY MEANS OF CARBON TETRACHLORIDE

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Carbon tetrachloride is often used to produce lesions of the liver tissue in animals [1, 2, 4]. Animals differ in their sensitivity to this compound. Rabbits, for example, possess high sensitivity to carbon tetrachloride, and this can be a serious obstacle to the production in these animals of frank cirrhosis of the liver: The animals often die before sclerosis of the organ develops. It is therefore important to develop methods of rendering rabbits susceptible to the action of carbon tetrachloride which will lead to the gradual development of significant cirrhotic changes in the liver tissue, reaching the stage of appearance of ascites and splenomegaly.

The technique which we recommend consists of the administration of carbon tetrachloride to rabbits in the form of separate courses, in the intervals between which the animals remain untreated in any way. Carbon tetrachloride is given to the rabbits twice a week, subcutaneously, in a dose of 0.2 ml (a 40% solution in peach oil) per kg body weight. After the first 12-14 injections an interval of 40-50 days is given, and this is followed by a second course of 8-10 injections, and then another interval, slightly longer than the first, and so on. The alternation of courses of injections of carbon tetrachloride with periods when no injections are given is controlled throughout the entire experiment by the protein fractions of the blood serum, for according to data in the literature and to previous research by one of us [3], an increase in the γ-globulins and a decrease in the serum albumins are associated with anatomical changes in the liver tissue in the process of development of cirrhosis. We investigated the serum protein fractions by the paper electrophoresis method, on the average, once every 10 days. The results showing the course of the changes in the serum protein fractions in the rabbits throughout the experiment are given in Fig. 1.

It may be seen from the curves that, after the first course of injections (1-1.5 months), a significant increase takes place (from 40 to 80%) in the serum γ-globulins, and an appreciable decrease (from 7 to 30%), in the albumin content. In the interval between the first and second courses of administration of carbon tetrachloride, a decrease in the γ-globulins and a slight increase in the albumins are observed, but in one rabbit, in which the least deviation from normal followed as a result of the first course of injections, a return to the original values was observed.

The second course of injections of CCl_4 is accompanied by a second sharp increase in the γ-globulins and a fall in the albumins; when this period again is followed by an interval, however, a clear tendency is once more found towards normalization of the serum protein fractions. A third course of carbon tetrachloride

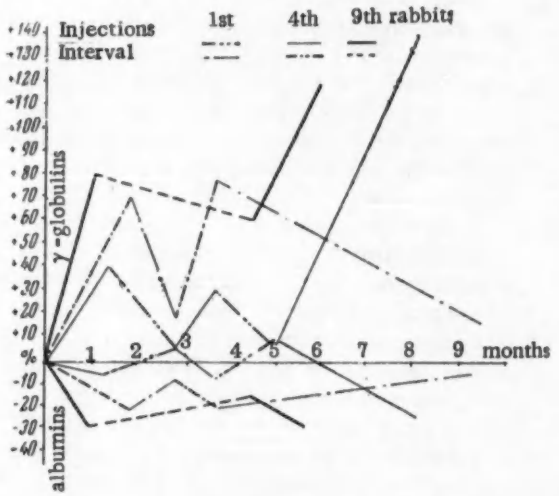

Fig. 1. Course of the changes in the serum protein fractions in rabbits during periods of administration of carbon tetrachloride and in the intervals between injections.

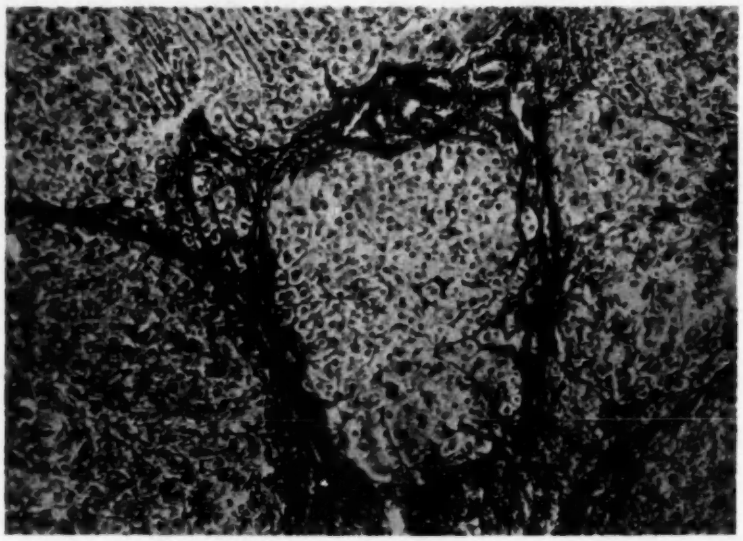

Fig. 2. Liver biopsy of a rabbit 8 months after the beginning of the experiment (3 courses of injections and 2 intervals). Well-marked annular cirrhosis of the liver.

injections, given to one of the experimental animals, gave the sharpest increase in the γ-globulins and the most significant fall in the albumins.

The administration of carbon tetrachloride thus causes considerable changes in the protein composition of the blood serum; when the action of the pathogenic factor is discontinued, however, these changes are partially reversed. The reparative processes in the periods between injections prevent the early death of the animals, as is observed after the continuous administration of carbon tetrachloride, and it enables the development of a more slowly progressive cirrhosis. It must be mentioned that the longer the duration of the experiment, the shorter must be the injection periods and the longer the intervals between them.

As a control of the morphological changes we performed laparotomy on animals after every 2-3 months, examined the liver and removed a piece of tissue for histological investigation. Frank cirrhosis, accompanied in certain cases by considerable enlargement of the spleen and ascites, could be observed in all animals without exception after 6-8 months of the experiment. On histological examination of the liver, an abundant proliferation of connective tissue composed of coarse fibers and a great decrease in hepatic tissue were observed (Fig. 2). Against this background, on the surface of the organ appeared multiple nodules, 0.4-0.5 cm in diameter. These nodules, being regenerative formations, gradually increased in size during the experiment.

It should be emphasized that when the experiment was performed in this way, in spite of the well-marked changes in the liver tissue, the animals remained for a long time in a completely satisfactory condition. This method may thus be used for the trial of various therapeutic measures in cirrhosis of the liver at various stages.

SUMMARY

To prevent an early death of animals with artificially induced cirrhosis of the liver it is suggested that carbon tetrachloride should be administered not continuously, as is usually done, but in separate courses, interrupted by periods free of injections. The protein fractions of the blood are periodically determined and biopsy is performed to control the condition of the liver. Cirrhosis of the liver, in a number of cases accompanied by ascitis and splenomegaly, develops in all the animals in 7 to 8 months.

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RUSSIAN JOURNALS FREQUENTLY CITED
[Biological Sciences]

Abbreviation*	Journal*	Translation
Agrobiol.	Agrobiologiya	Agrobiology
Akusherstvo i Ginekol.	Akusherstvo i Ginekologiya	Obstetrics and Gynecology
Antibiotiki	Antibiotiki	Antibiotics
Aptechnoe Delo	Aptechnoe Delo	Pharmaceutical Transactions
Arkh. Anat. Gistol. i Émbriol.	Arkhiv Anatomii Gistologii i Émbriologii	Archives of Antomy, Histology, and Embryology
Arkh. Biol. Nauk SSSR	Arkhiv Biologicheskikh Nauk SSSR	Archives of Biological Science USSR
Arkh. Patol.	Arkhiv Patologii	Archives of Pathology
Biofizika	Biofizika	Biophysics
Biokhimiya	Biokhimiya	Biochemistry
Biokhim. Plodov i Ovoshchei	Biokhimiya Plodov i Ovoshchei	Biochemistry of Fruits and Vegetables
Bot. Zhur.	Botanicheskii Zhurnal	Journal of Botany
Byull. Éksptl. Biol. i Med.	Byulleten Éksperimentalnoi Biologii i Meditsiny	Bulletin of Experimental Biology and Medicine
Byull. Moskov. Obshchestva Ispytatelei Prirody, Otdel Biol.	Byulleten Moskovskogo Obshchestva Ispytatelei Prirody, Otdel Biologicheskii	Bulletin of the Moscow Naturalists Society, Division of Biology
Doklady Akad. Nauk SSSR	Doklady Akademii Nauk SSSR	Proceedings of the Academy of Sciences USSR
Éksptl. Khirurg.	Éksperimentalnaya Khirurgiya	Experimental Surgery
Farmakol. i Toksikol.	Farmakologiya i Toksikologiya	Pharmacology and Toxicology
Farmatsiya	Farmatsiya	Pharmacy
Fiziol. Rastenii	Fiziologiya Rastenii	Plant Physiology
Fiziol. Zhur. SSSR	Fiziologicheskii Zhurnal SSSR im. I. M. Sechenova	I. M. Sechenov Physiology Journal USSR
Gigiena i Sanit.	Gigiena i Sanitariya	Hygiene and Sanitation
Izvest. Akad. Nauk SSSR, Ser. Biol.	Izvestiya Akademii Nauk SSSR, Seriya Biologicheskaya	Bulletin of the Academy of Sciences USSR, Biology Series
Izvest. Tikhookeanskogo N. I. Inst. Rybnogo Khoz. i Okeanog.	Investiya Tikhookeanskogo N. I. Instituta Rybnogo Khozyaistva i Okeanografii	Bulletin of the Pacific Ocean Scientific Institute of Fisheries and Oceanography
Khirurgiya	Khirurgiya	Surgery
Klin. Med.	Klinicheskaya Meditsina	Clinical Medicine
Lab. Delo	Laboratornoe Delo (po Voprosam Meditsiny)	Laboratory Work (on Medical Problems)
Med. Parazitol.	Meditsinskaya Parazitologiya i Parazitarnye Bolezni	Medical Parasitology and Parasitic Diseases
Med. Radiol.	Meditsinskaya Radiologiya	Medical Radiology
Med. Zhur. Ukrain.	Medichnii Zhurnal Ukrainskii	Ukrainian Medical Journal
Mikrobiologiya	Mikrobiologiya	Microbiology
Mikrobiol. Zhur.	Mikrobiologichnii Zhurnal	Microbiology Journal
Nevropatol., Psikhyat. i Psikhogig.	Nevropatologiya, Psikhyatriya i Psikhogigiena	Neuropathology, Psychiatry and Psychohygiene
Ortoped., Travmatol. i Protez.	Ortopediya, Travmatologiya i Protezirovanie	Orthopedics, Traumatology and Prosthetics
Parazitol. Sbornik	Parazitologicheskii Sbornik	Parasitology Collection
Pediatriya	Pediatriya	Pediatrics
Pochvovedenie	Pochvovedenie	Soil Science
Priroda	Priroda	Nature
Problemy Éndokrinol. i Gormonoterap.	Problemy Endokrinologii i Gormonoterapii	Problems of Endocrinology and Hormone Therapy
Problemy Gematol.	Problemy Gematologii i Perelivaniya Krovi	Problems of Hematology and Blood Transfusion
Problemy Tuberk.	Problemy Tuberkuleza	Problems of Tuberculosis
Sovet. Med.	Sovetskaya Meditsina	Soviet Medicine
Sovet. Vrachebny Zhur.	Sovetskii Vrachebnyi Zhurnal	Soviet Physicians Journal
Stomatologiya	Stomatologiya	Stomatology

* BRITISH-AMERICAN TRANSLITERATION SYSTEM.

(continued)

(continued)

Abbreviation	Journal	Translation
Terap. Arkh.	Terapevticheskii Arkhiv	Therapeutic Archives
Trudy Gelmint. Lab.	Trudy Gelmintologicheskoi Laboratoriya	Transactions of the Helminthology Laboratory
Trudy Inst. Genet.	Trudy Instituta Genetiki	Transactions of the Institute of Genetics
Trudy Inst. Gidrobiol.	Trudy Instituta Gidrobiologiya	Transactions of the Institute of Hydrobiology
Trudy Inst. Mikrobiol.	Trudy Instituta Mikrobiologiya	Transactions of the Institute of Microbiology
Trudy Inst. Okean.	Trudy Instituta Okeanologiya, Akademii Nauk SSSR	Transactions of the Institute of Oceanology, Academy of Sciences, USSR
Trudy Leningrad Obshchestva Estestvoisp.	Trudy Leningrad Obshchestva Estestvoispytatelei	Transactions of the Leningrad Society of Naturalists
Trudy Vsesoyuz. Gidrobiol. Obshchestva	Trudy Vsesoyuznogo Gidrobiologicheskogo Obshchestva	Transactions of the All-Union Hydrobiological Society
Trudy Vsesoyuz. Inst. Ekspti. Med.	Trudy Vsesoyuznogo Instituta Eksperimentalnoi Meditsiny	Transactions of the All-Union Institute of Experimental Medicine
Ukrain. Biokhim. Zhur.	Ukrainskii Biokhimichnii Zhurnal	Ukrainian Biochemical Journal
Urologiya	Urologiya	Urology
Uspekhi Biokhimiya	Uspekhi Biokhimiya	Progress in Biochemistry
Uspekhi Sovremennoi Biol.	Uspekhi Sovremennoi Biologiya	Progress in Contemporary Biology
Vestnik Akad. Med. Nauk SSSR	Vestnik Akademii Meditsinskikh Nauk SSSR	Bulletin of the Academy of Medical Science USSR
Vestnik Khirurg. im. Grekova	Vestnik Khirurgii imeni Grekova	Grekov Bulletin of Surgery
Vestnik Leningrad. Univ. Ser. Biol.	Vestnik Leningradskogo Universiteta, Seriya Biologii	Journal of the Leningrad Univ., Biology Series
Vestnik Moskov. Univ., Ser. Biol. i Pochvov.	Vestnik Moskovskogo Universitera, Seriya Biologii i Pochvovedeniya	Bulletin of the Moscow University, Biology and Soil Science Series
Vestnik Oftalmol.	Vestnik Oftalmologii	Bulletin of Ophthalmology
Vestnik Oto-rino-laringol.	Vestnik Oto-rino-laringologiya	Bulletin of Otorhinolaryngology
Vestnik Rentgenol. i Radiol.	Vestnik Rentgenologii i Radiologii	Bulletin of Roentgenology and Radiology
Vestnik Venerol. i Dermatol.	Vestnik Venerologii i Dermatologii	Bulletin of Venereology and Dermatology
Veterinariya	Veterinariya	Veterinary Science
Vinodelie i Vinogradarstvo	Vinodelie i Vinogradarstvo SSSR	Wine-Making and Viticulture
Voprosy Klin.	Voprosy Klinicheskie	Clinical Problems
Voprosy Med. Khim.	Voprosy Meditsinskoi Khimii	Problems of Medical Chemistry
Voprosy Med. Virusol.	Voprosy Meditsinskoi Virusologii	Problems of Medical Virology
Voprosy Neirokhirurg.	Voprosy Neirokhirugii	Problems of Neurosurgery
Voprosy Onkol.	Voprosy Onkologii	Problems of Oncology
Voprosy Pitaniya	Voprosy Pitaniya	Problems of Nutrition
Voprosy Psikhologii	Voprosy Psikhologii	Problems of Psychology
Voprosy Virusologii	Voprosy Virusologii	Problems of Virology
Vrachebnoe Delo	Vrachebnoe Delo	Medical Profession
Zav. Lab.	Zavodskaya Laboratoriya	Factory Laboratory
Zhur. Mikrobiol., Epidemiol. i Immunobiol.	Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii	Journal of Microbiology, Epidemiology, and Immunobiology
Zhur. Nevropatol. i Psikhiat.	Zhurnal Nevropatologii i Psikhiatrii imeni S. S. Korsakov	S. S. Korsakov Journal of Neuropathology and Psychiatry
Zhur. Obshchei Biol.	Zhurnal Obshchei Biologiya	Journal of General Biology
Zhur. Vysshei Nerv. Deyatel.	Zhurnal Vysshei Nervnoi Deyatelnosti imeni I. P. Pavlova	I. P. Pavlov Journal of Higher Nervous Activity
Zool. Zhur.	Zoologicheskii Zhurnal	Journal of Zoology

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